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Phytopathology

An International Journal

Official Organ of
The American Phytopathological Society

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LEIGH HUMBOLDT PENNINGTON

PHYTOPATHOLOGY

VOLUME 22 NUMBER 11

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LEIGH HUMBOLDT PENNINGTON
1877–1929

PERLEY SPAULDING

Doctor Leigh H. Pennington died suddenly from heart disease on April 24, 1929, while in Washington, D. C., carrying on investigations of the white-pine-blister rust for the Federal Bureau of Plant Industry during sabbatical leave. He had worked the day before and until 10 o'clock at night at his office, apparently in normal health. When he did not appear at the usual time in the morning investigation revealed his death. He was 51 years of age. He leaves his wife and one daughter, Mrs. Phyllis Percival.

Doctor Pennington was a son of Claribel (Pratt) and Byron Humboldt Pennington. He was born October 26, 1877, in the crossroads hamlet of Pennington's Corners in the town of Macon, Michigan. The family lived in the original home of his grandfather. The grandfather, Israel Pennington, moved about 1820 from Farmington, near Canandaigua, Ontario County, New York, to Pennington's Corners. The hamlet evidently took its name from this family, members of which even yet occupy the land in that locality, although it is owned by Henry Ford, who has preserved an old-time community in all of the original simplicity that is now possible. There, quite a large nursery was established and maintained by the grandfather. At the death of its original owner the father continued the business and a large part of it still stands on the original location. The grandfather was a great student, reciting poetry while at work in the field and making a real study of his work. The mother taught in a seminary until her marriage. The father was a civil engineer, graduating at the University of Michigan. The family was thus well above the average in education and was in a leading position in its community. The boy very naturally studied plants and adopted teaching as a livelihood. Experience about the nursery and more or less conscious imitation of his father undoubtedly trained him in the habit of carefully noting details. His memory was excellent, so that he depended upon it more than most people can. In the end, this was unfortunate, as it resulted in much of his last work being lost because of lack of records of things perfectly well known to him but unknown to any one else.

His early training was in the public schools of Macon and high school of Tecumseh, Michigan. Later he taught in the public schools of Macon

and near-by towns for 3 years. He was principal in the Palmyra High School for 5 years. At this time he met his future wife, Miss Blanche Van Fleet. They were married in 1902. In 1904 he entered the University of Michigan and received the degree of A. B. 3 years later. He at once began post-graduate work with Doctor Newcombe at the same university, being given the degree of Doctor of Philosophy in Botany in 1909. During the last 4 years he was assistant in the Department of Botany. One year was spent as instructor in botany at Northwestern University, after which he went to Syracuse University as Assistant Professor of Botany in the College of Liberal Arts. Two years later he became Associate Professor. In 1914 a Department of Forest Botany was established in the New York State College of Forestry of Syracuse University and he was made Head Professor, which post he held until his death.

During the summer vacations of the college Doctor Pennington occupied his time with investigations of forest-pathological problems. He served as expert in the United States Department of Agriculture in the summers of 1911 and 1912; collaborator of the New York Botanical Garden in 1913 and 1914; since 1917 as pathologist or collaborator with the United States Department of Agriculture, engaged in studies of the white-pine-blister rust, specializing in its epiphytology. These studies continued until his death. One entire sabbatical year was thus spent in British Columbia.

Doctor Pennington was a fellow of the American Association for the Advancement of Science, member of the Botanical Society of America, The American Phytopathological Society, the American Ornithologists Union, the American Genetics Association, the Society of American Foresters, the New York State Forestry Association, and the fraternities Sigma Xi, Phi Kappa Phi, and Alpha Xi Sigma. He was a 32nd degree Mason. He was active in the University Methodist Church and was interested in many philanthropies. He always tithed his income. He was constantly helping students out of their difficulties, being exceedingly liberal of his time, interest, and money.

Doctor Pennington was most devoted to his family, a progressive member of his community, and a loyal and devout member of his church. He was characterized, above all, by his sterling honesty and common sense. Whatever the situation, he instinctively chose the sound plan of action. Clear thinking naturally resulted in the logical and best action. Like many strong thinkers, he was very unassuming. When an opinion was once formed it was held tenaciously unless his opponent could convince him that he had formed it on false premises. He was a staunch friend when one once really penetrated his somewhat austere exterior. He was a successful teacher, beloved by his students to an unusual degree. He was always ready to

help his boys and had their confidence and good-will. Had he chosen to push himself to the front, he undoubtedly could have been successful in a much more prominent way, but that would have been distasteful to him in the highest degree. In all his various activities he chose some main objective and strove for it, ignoring trifles and distractions. His counsel was sought and heeded both by students and in matters of policy of the college.

In his profession he was an intensely hard worker, although his unhurried manner would often belie that fact. He was a keen observer of human nature, a thorough student, and an excellent scientific investigator. He had a keen sense of humor that was shown on occasion by quiet chuckles rather than gales of laughter. He was meticulously careful of details, possibly due to an incident of his early youth. On learning that his boy was collecting birds' eggs, his father required him to record properly all the collection data for each set taken so that the collection would have real scientific value. He was strong in initiative and sometimes persisted in a line of action almost to the point of foolhardiness, it seemed to his friends. But, with all this, he was resourceful and self-reliant, so he probably did not overstep the limits of his abilities.

His absolute sincerity gave him an attractive personality to those who look beneath the surface. His reserved manner, resulting from his strong self-control and equanimity, sometimes threw people off the track when they first met him. His pronounced initiative made him an excellent leader on expeditions of all sorts. The writer has accompanied him on trips of different lengths and difficulty; has, with him, listened to the courting song of an approaching black bear in the Adirondacks at dusk; inspected newly made bear tracks, not yet filled with muddy water, in grizzly-bear country in British Columbia; and the same self-possession and poise were always evident. The writer's first airplane trip was made with him as companion in the forward cockpit of a 4-man hydroplane on the coast of British Columbia. Hardly had the machine taken off in a heavy head wind before the engine died and a forced landing had to be made in the main channel with waves running several feet high. The force of the landing broke the slatted seat under him, but he turned not a hair. He suffered from terrific headaches occasionally and undoubtedly knew that a form of indigestion was the cause. He made considerable study of suitable foods and was a good camp cook for a small party. He knew the uses of native plants, especially mushrooms, and often eked out a limited larder on trips by using them.

Professionally, Doctor Pennington was considered one of the soundest workers in forest pathology. He prided himself on being the only full professor in forest pathology in the country. He was a good mycologist. In this as in other lines of work he realized that specialization was the order

of the day and made himself the authority on the genus Marasmius, publishing on this genus for temperate North America in "North American Flora." In the same methodical and thorough manner he worked several years on the white-pine-blister rust with respect to the time of its introduction into various regions of this continent, its rate of spread from various centers, its means of spreading, and its probable final range. Indeed, he was working on this problem at the time of his death. In accounting for its extensive and rapid spread in British Columbia his knowledge of the birds came into play, so that he was able to eliminate them as carriers of the disease over long distances.

He collected birds' eggs from boyhood with scientific care and accuracy. Exchanges and purchases made his one of the notable private collections in the country. He specialized on the Raptores and the birds of New York State. He also had a valuable library on ornithology. He knew the birds well for an amateur unable to devote much time to his hobby. While in British Columbia, studying the spread and past history of the white-pine-blister rust there, he made himself familiar with the birds, being there an entire year, so that he saw the migrating as well as the resident species. It was the writer's good fortune to see the spring migration of 1922 with him. With his assistance notes were made on the species observed, which were considered valuable by the U. S. Bureau of Biological Survey, as they were made in a region where such observations were lacking in their records.

He was also deeply interested in theology and New Testament translations. He was a keen collector of such books. Just before his death he was studying Greek so that he could read the New Testament in that language.

Such a man cannot be replaced in his profession, community, or church. Such a character is too rare in this imperfect world to be duplicated in the same community.

The circle is broken,—one seat is forsaken,—
One bud from the tree of our friendship is shaken,—

WORDSWORTH.

DIVISION OF FOREST PATHOLOGY,
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SOME PROBLEMS CONCERNING BLUE MOLD IN RELATION TO CLEANING AND PACKING OF APPLES[1]

KENNETH F. BAKER[2] AND F. D. HEALD[3]

(Accepted for publication March 14, 1932)

INTRODUCTION

During storage of the Washington apple crop each season much loss is sustained from diseases. Exact figures indicating the amount of this loss due to specific organisms are difficult to obtain. Pailthorp and Park (9) reported that an average of 21.3 per cent of the rejections of Washington car shipments in the crops of 1922–1925 on eastern terminals were due to decay. It is generally conceded that blue mold is the cause of the greater part of this loss. Brooks, Cooley, and Fisher (1) report that blue mold causes from 80 to 95 per cent of the total rots of the crop. Heald *et al.* (4) reported an average of 3.76 per cent blue-mold decay in 2,102 cars of the 1925 crop and 3.65 per cent in 1,795 cars in the 1926 crop on eastern terminals. Heald and Ruehle (5) found that condition reports of the 1929 Washington apple crop at the time of movement from local storage showed many cars with 2 to 3 per cent decay, a considerable number 3 to 7 per cent, and a smaller number 8 to 10 per cent, while some few lots showed from 12 to 40 per cent blue mold. They stated that at least 75 per cent of the storage rots in apples from the principal Washington districts are due to blue mold. Thom (12) and Heald and Ruehle (5) reported that *Penicillium expansum* Link is the most important blue-mold species in the cause of apple decay.

The passage of the Federal Food and Drug Inspection Act limited the arsenical load of apples for domestic sale in 1927 to 0.025 grain arsenous oxide per pound of apple, and this figure was reduced to 0.012 grain by 1931. Since the Act went into effect the extensive investigations on fruit-cleaning methods have shown that washing the fruit with various solvents is superior to other methods. As now employed these solutions contain either hydrochloric acid or some of the various alkaline compounds.

[1] Published with the approval of the Director of the Washington Agricultural Experiment Station as Scientific Paper No. 221, State College of Agriculture and Agricultural Experiment Station, Pullman, Washington.

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[3] The writers wish to acknowledge their indebtedness to Dr. J. L. St. John and Dr. G. A. Huber for helpful suggestions and assistance during the progress of this investigation.

Hydrochloric acid is more commonly employed because of its cheapness, ease of use, and slight danger of injury to fruit. However, in the 1931 season a number of packing plants changed to alkaline washes in order to reduce the arsenical deposits to the tolerance. These solutions have been commonly used in the 1930 and 1931 seasons at temperatures up to 110° F. and sometimes 120° F. in order to facilitate cleaning. It is the experience of growers and investigators (Diehl *et al.*, 2; Heald and Ruehle, 5) that temperatures of 120° F. or more tend to bleach fruit and may lower the keeping quality.

As early as 1907 Smith (10) found that the washing tanks were one of the main sources of infection of brown rot of lemons and tested various disinfectants to reduce the spore load of the tanks. It was later found that other decays of citrus fruit were spread in a similar manner. Various compounds have been employed in the citrus industry in an attempt to counteract the effect of this inoculum bath and decrease the amount of decay. For a time borax was used, but, since 1927, sodium bicarbonate has been employed.

Heald *et al.* (4) found that the apple-washing tank was one of the main sources of contamination of apples and that an increase of the spore load increased the percentage of decay. Treating the tanks during the night with a formaldehyde solution to keep the spore load reduced from day to day was recommended. This has since been recommended by other workers but has not been generally adopted.

No practical treating solution for the control of blue mold of apples in a manner comparable to that in use in the citrus industry for blue and green molds has yet been found. The similarity of the two washing treatments and the fact that it does reduce decay in citrus fruits have led firms selling various washing compounds to claim that these substances greatly reduce blue mold of apples and that they are toxic to *Penicillium expansum* spores. Fisher and Reeves (3) reported that the washing solutions have little or no toxicity to fungous spores, and Diehl *et al.* (2) reported that spores of *Gloeosporium perennans* Zeller and Childs were not killed by 6 days' exposure to either acid or alkaline washing solutions.

The extreme resistance of the spores of Penicillium species to various agents has long been known. Various species have been reported growing on strong copper sulphate solutions and concentrated sugar solutions. Of the fungi studied, Stevens (11) found that *P. crustaceum* Fries was the most resistant to acids, alkalies, and salts.

The purpose of this investigation was to determine the degree of toxicity of 3 of the most commonly used washing solutions to spores of *Penicillium expansum* and to investigate some of the factors that would be involved in

the use of a treating solution for lessening decay. A practical method of reducing the spore load of some of the sources of contamination of the washing tank was examined.

MATERIALS AND METHODS

In this investigation the cultures used were transfers from the Penicillium[4] found by Heald and Ruehle (5) to be most abundant in occurrence and to cause the most rapid decay of apples. Unless otherwise mentioned the spores used in this work were taken from 5- to 9-day-old, vigorously growing plates of *P. expansum*, and no old dried cultures or young plates just beginning to fruit were employed.

Spore suspensions were made by placing spores scraped from the surface of the Petri-dish cultures of the organism into a flask containing 300 cc. sterile distilled water and shaking vigorously for 5 minutes to break up the spore clumps. Five cc. of this suspension were transferred with a sterile pipette to flasks containing 300 cc. sterile distilled water plus the desired quantity of the compound whose toxicity was being tested, and shaken for 5 minutes. Before each flask was to be tested for spore viability it was thoroughly shaken to insure uniformity of spore suspension. Unless otherwise stated 1 cc. of this suspension was transferred with a sterile pipette to each of three 9-cc. sterile water blanks, and these were simultaneously shaken. One cc. of each of these 1 to 10 dilutions was transferred with a sterile pipette to sterile Petri dishes. Two per cent potato dextrose agar cooled to about 42° F. was poured over this transfer and the dish rotated. In the early series the cheek test of estimation of the temperature was employed. In later series, in order to eliminate the temperature variable, the agar was melted and allowed to cool nearly to the solidifying point. It was then placed in an incubator maintained at a temperature of 42° F. for from 5 to 12 hours in order to standardize the temperature. No more fluctuation occurred in tests on media of estimated temperature than on those of known temperature. Plates were incubated at room temperature. When the colonies first began to show green coloration counts were made by inverting the plates over a Spencer substage lamp. The entire plate was counted in sections outlined with wax pencil. Thus the figures in the tables in this paper are averages of the 3 plates made from each flask at each viability test and are expressions of the approximate number of viable spores per cubic centimeter in the solution at the time the test was taken.

During the 1930 and 1931 seasons packing plants used hydrochloric acid at a strength of 1 to 3 per cent by volume and kept it at the desired acidity by titration. In this study commercial grade HCl was used at 3 per cent

[4] Identified by Dr. Charles Thom as *Penicillium expansum* Link.

by volume, or, in terms of the experiment, 9 cc. of concentrated commercial HCl to a 300 cc. water blank. Measured by the hydrogen-electrode method, this gave a solution of pH 1.2.

The commercial cleaner of the sodium carbonate-borax type[5] was reported by the company to consist of 100 parts of soda ash to 1 part of borax in the 1930 season. It was used at the rate of ½–1½ lb. per gallon of water. Technical caustic soda flakes were added in cases where the fruit was difficult to clean, and then never over ½ oz. per gallon of solution. The ingredients are shipped separately to the packing plant and mixed as used. The cleaner was used in this study at the rate of 1½ lb. per gallon (without caustic soda, as this substance was rarely used in washing operations). Materials used were the soda ash and borax supplied by the company from their commercial stock. In terms of the study the strength was 53.91 gm. per 300 cc. water blank, giving a solution with a pH value of 10.3 measured by the hydrogen electrode.

The commercial cleaner of the sodium carbonate-trisodium phosphate type[6] was reported as composed of equal parts of each constituent and used at the rate of 60 lb. per 100 gal. of water in the 1930 season. In 1931 it was used in varying proportions at greater strengths, one case of 300 lb. per 250 gal. being known. The ingredients of this cleaner are mixed at the factory. Material used in this study was the stock mixed powder supplied by the company. In terms of the experiment the strength was 21.5 gm. per 300 cc. water blank. The solution measured pH 10.3 by the hydrogen-electrode method.

In order to obtain the effect of the solutions at different temperatures the flasks were placed in an incubator, the temperaure of which did not fluctuate more than 2.5° F. in 1 week. The flasks were placed in pans of water to stabilize the temperature and to reduce the evaporation of the solutions that would have increased the concentration of the treating compound.

The temperature of the flasks was stabilized by keeping them in an incubator for 10 to 12 hours at the desired degree, after which the spores were added. Transfers from the flasks were made in a sterile culture chamber and usually required not more than 10 minutes. The temperature fluctuation of the solutions was slight during that time.

Time required for germination, percentage of germination, amount of growth of the germ tube in a given time, and other criteria have been used as measures of toxicity of substances by other workers. As the spores are in suspension in the solutions in the washing tanks and are directly sub-

[5] Brogdite cleaner, supplied by the Northwest Brogdex Co., Yakima, Washington.

[6] Laux cleaner, supplied by The Laux Laboratories, Seattle, Washington.

jected to both chemical toxicity and temperature factors, it was deemed advisable to duplicate as nearly as possible these conditions in the tests. A delaying of germination due to one or both factors involved is of no great importance in the ultimate fruit decay because of prolonged storage periods. The criterion of spore viability adopted was the ability to form colonies on 2 per cent dextrose potato-agar.

The degree of experimental variation with this technique was determined (Table I). Two flasks of 300 cc. sterile distilled water were inoculated

TABLE 1.—*Number of viable spores of blue mold per cubic centimeter obtained in replicated plates from the same spore suspension*

Plate	Flask 1	Flask 2	
		A Series	B Series
1	3,430	2,250	.....
2	3,700	2,460	.....
3	3,700	2,330	.....
4	4,710	2,150	.....
5	4,650	2,160	.....
6	3,660	2,200	.....
7	3,570	2,470	.....
8	3,500	2,180	.....
9	3,330	2,260	2,250
10	3,140	2,750	2,540
11	3,250	2,100	2,140
12	3,990	2,010	2,240
13	3,320	2,430	2,130
14	3,200	2,160	2,120
15	3,550	2,100	2,240
16	3,130	2,220	2,110
17	3,210	2,340	2,180
18	2,470	2,080	2,170
19	4,270	2,330	2,120
20	2,990	2,180	2,060
Average of series	3,523	2,258	2,191

with a spore suspension and 20 plates made at once from each. The A and B series of flask 2 show the possible variations of 2 plates made from the same dilution tube with the same pipette.

When high spore concentrations were employed the range of experimental variation was fairly broad, and, hence, only large constant changes in number of viable spores per cubic centimeter are significant in the experiment. Within limits, however, there is considerable uniformity of results.

EFFECTS OF SPORE AGE ON RESISTANCE TO HEAT AND TOXIC COMPOUNDS

The factor of spore age has been shown to have some effect on resistance to both temperature and toxic substances. Henderson Smith (6) found that the resistance of Botrytis spores to phenol increased with the age, and Marloth (8), using alkaline solutions, states, "The resistance of 60-day-old spores against the treating substances as compared with 14-day-old is quite marked for *P. digitatum.*" The effect of spore age on resistance to the temperature factor in these experiments is shown in table 2. Two flasks of 300 cc. sterile distilled water were inoculated with 5 cc. of a suspension of spores from 5-day-old and 49-day-old cultures, respectively. These were incubated at 110° F. and plates made at stated intervals.

TABLE 2.—*Number of viable spores of blue mold per cubic centimeter obtained at intervals from water suspensions of young and old spores incubated at 110° F.*

Time	Flask with 49-day-old spores	Flask with 5-day-old spores
0 hours	2,376	2,426
1 hour later	1,200	990
2 hours "	1,426	1,020
3 " "	1,600	1,170
4 " "	1,490	1,460
5 " "	1,050	1,286
6 " "	903	1,180
7 " "	826	980
8 " "	743	896
10 " "	133	106
12 " "	106	2

It appears that there is no significant difference in resistance to temperature as a result of differing age.

The effect of spore age on resistance to the treating solutions is shown in table 3. Two 300 cc. sterile distilled water blanks containing 3 per cent by volume of commercial hydrochloric acid were inoculated with suspensions of spores from 5-day-old and 54-day-old cultures. Petri-dish transfers from these flasks were made at indicated intervals. The results indicated that older spores were considerably more resistant to hydrochloric acid than those more recently formed, paralleling the results of Marloth (8) with alkaline solutions and Henderson Smith (6) with phenol.

The resistance of 9-day-old and 44-day-old spores to a hypochlorite solution was determined (Table 4). Sodium hypochlorite solutions were found, in general, to have a high toxicity to *Penicillium expansum* spores. A

TABLE 3.—*Number of viable spores of blue mold per cubic centimeter obtained at intervals from suspensions of old and young spores in acid cleaner at room temperature*

Time	54-day-old spores	5-day-old spores
0 hours	2,916	5,180
4¼ " later	3,363	4,020
9¼ " "	3,470	2,770
18½ " "	3,190	1,703
26 " "	3,033	990
34¾ " "	2,616	396
48 " "	2,330	76
70½ " "	973	0
96 " "	33	0
126 " "	0	0

commercial solution of this type[7] (testing 5.7 per cent available chlorine by the acetic acid method against a standard sodium thiosulphate solution) was used at the rate of 1 cc. to a 300 cc. sterile distilled water blank. Triplicate flasks held at room temperature were used for each series and plates made in triplicate from each at indicated intervals.

TABLE 4.—*Number of viable spores of blue mold per cubic centimeter at intervals in water suspension of young and old spores plus hyopchlorite solution*

Time	9-day-old spores			44-day-old spores		
	Flask 1	Flask 2	Flask 3	Flask 1	Flask 2	Flask 3
0 min.	2,660	2,680	2,700	2,360	2,480	2,750
2½ "	1,960	2,190	2,790	2,280	2,410	2,680
5 "	2,990	2,510	2,630	2,350	2,460	2,530
7½ "	2,830	2,650	2,640	2,400	2,380	2,490
10 "	1,450	2,670	2,710	2,330	2,310	2,660
12½ "	80	320	530	2,210	2,050	2,700
15 "	80	230	440	940	1,660	2,640
17½ "	60	110	40	280	290	750
20 "	50	120	150	100	110	400

The resistance of blue-mold spores to the toxic substances tested increased with the aging of the spores, probably because of lowered water content, changes in the protoplasm and cell wall and retarded life processes.

Five-day-old and 9-day-old spores were placed in 300 cc. water blanks plus 3 per cent commercial hydrochloric acid and held at room temperature. Variation in the lethal action due to the 4-day difference in age did not

[7] Clorox, manufactured by Clorox Chemical Co., Oakland, California.

exceed the normal experimental error. Any group of spores obtainable would also be of varying ages due to the manner of their development on the conidiophores.

THE RELATION OF pH OF MEDIUM TO THE GROWTH OF COLONIES

The hydrogen-ion concentration of dilution water blanks and of poured plates made from them was determined by the colorimetric method (Table 5).

TABLE 5.—*The pH of 1–10 dilution tubes and corresponding Petri-dish cultures when cleaning mixtures are added*

Solution	pH concentration	
	1–10 dilution tube	Petri dish
Sodium carbonate-borax cleaner, 1½ lb. per gal.	above 9.6	8.2
Sodium carbonate-trisodium phosphate cleaner, standard solution	above 9.6	6.6
Hydrochloric acid, 3 per cent solution	below 1.8	5.3

As the pH of ordinary 2 per cent dextrose potato-agar is about 6.2 these fluctuations from the treating solutions might have contributed to variations in the results. As no information on the relation of hydrogen-ion concentration to growth of *Penicillium expansum* was found in the literature, this factor was considered. The shortcomings of the system of growing an organism on plate cultures to determine its pH relations (buffer action of the medium and the autolytic action of the organism), as pointed out by Marloth (8), are realized. It seemed desirable to use the following method, however, as the organism was being grown in plates in the experiment. Two per cent dextrose potato-agar was, accordingly, titrated by the colorimetric method, when either a dilute HCl or NaOH solution was added to obtain the different H-ion concentrations. A single, nonsporulating, minute colony of *P. expansum* developed from spores thinly scattered on 2 per cent dextrose potato-agar plates was planted in the center of each Petri dish. The plates were incubated at room temperature. At pH 2.3 the medium was completely liquefied, so that normal growth did not occur. The measurements in centimeters across the greatest diameters of the colonies at the end of 7 days are given in table 6. Apparently the growth of the organism was not greatly affected by the hydrogen-ion concentration within the ranges tested. For this reason little fluctuation in growth occurred from the changes in pH of the medium from the addition of treating solutions to the spore suspension.

TABLE 6.—*The diameter in centimeters of colonies of blue mold after 7 days on media of different pH values*

Plate	pH 2.3	3.8	4.6	5.5	6.2	7.0	8.0	9.6
1	1.5	5.3	5.2	5.3	5.0	4.9	4.8	4.7
2	1.6	5.4	5.2	5.1	5.1	4.9	4.4	4.8
3	1.6	5.0	5.0	5.2	5.1	4.9	.....	4.9
4	1.5	4.8	5.3	5.3	5.1	5.2	.....	4.8
Average	1.55	5.12	5.17	5.25	5.07	4.75	4.6	4.8

THE RELATION OF TEMPERATURE TO SPORE GERMINATION

In order to determine the effect of different temperatures on spore germination, tests were conducted with sterile distilled water, tap water, and 2 per cent apple juice in duplicate. The pH values of these solutions were 7.9, 8.6, and 4.3, respectively. No germination had occurred at the end of 2 weeks in the tap water and distilled water incubated at room temperature and at 90° F. The spores germinated readily when plated out with 2 per cent dextrose potato-agar. When 2 per cent apple juice was used no germination occurred in 14 days at 90° F.; but, at room temperature, there was no germination in 4 hours, 15 per cent in 12 hours, 20 per cent in 27½ hours, 25 per cent in 37½ hours, 35 per cent in 47½ hours, 40 per cent in 76 hours, 40 per cent in 126½ hours, and 40 per cent in 14 days. This low germination was due to the extreme spore concentrations used. Spores from these flasks germinated readily when plated out with 2 per cent dextrose potato-agar at the end of 14 days.

Examination of the spores in the flasks of the washing solution showed in all cases that they had not germinated. Any toxic action exerted by the solutions tested is, therefore, on the spores themselves and not on the germ tubes.

The germination of *Penicillium expansum* spores at 32° F. was determined. Eight-day-old spores were placed in 50-cc. flasks containing 25, 50, and 100 per cent, respectively, of sterile apple juice. One flask of each was incubated at room temperature, and 2 were held in an electric refrigerator at 32° F. In 12 hours some germination was noted in the 25 and 50 per cent solutions held at room temperature. In 23, 48, and 72 hours germination was evident in all the flasks held at room temperature, but none in those at 32° F. At the end of 7 days germination was noted in all the flasks at 32° F., though the germ tubes were small.

This experiment was repeated. Brown's synthetic medium was used in the place of the apple juice. At room temperature abundant germination occurred in 20 hours, and a few spores had germinated in the solutions at 32° F. in 172 hours.

Spores from 9-day-old cultures were frozen in a 10 per cent sterile apple-juice solution and held in an electric refrigerator at 21°–25° F. for 101 hours. The outer portion of the ice was melted quickly and the ice cake washed several times with sterile distilled water to remove any spores from the sides of the flask that were not frozen in the ice. The flasks were held at room temperature. Examination of the solution 36 hours after melting showed germination of the spores, the percentage being nearly as great as in the check held at room temperature.

THE EFFECT OF CLEANING SOLUTIONS ON BLUE-MOLD SPORES AT DIFFERENT TEMPERATURES

The commercial practice is to heat the washing tanks to 100–110° F. during operation and then to shut off the heat during the night. It has been observed that the temperatures of these tanks drop during the night from the initial temperature of 100 to 110° F. at 6:00 p. m. to 70 to 85° F. at 4:30 a. m., depending on the exposure of the machines and the temperature of the air. The heat is turned on again in the early morning, bringing the solution to the operating temperature again in time to begin operations at 7:00 a. m. One would suppose from the work reported in this paper that these conditions would greatly reduce the spore load of the tanks. In order to test this supposition bottles of the treating solution were taken from a machine in Wenatchee, Washington, employing 1 per cent acid and a machine using sodium carbonate-trisodium phosphate cleaner (300 lb. to 250 gal.). Samples were taken at the end of the day's run, before the sediment had settled. On the following day, before beginning the test, these solutions were incubated at 110° F. for several hours to stabilize their temperature. The solutions were inoculated with a suspension of blue-mold spores and plates made in triplicate, 1–300 dilution being used for the acid and 1–18,000 for the alkaline wash (to reduce the inhibiting action of the cleaner). They were then returned to the incubator and the temperature lowered gradually to 72° F. in 11½ hours, a thermograph record being kept. Plates were made again at the end of that period (Table 7).

TABLE 7.—*Effect of the overnight stand on the number of viable blue-mold spores per cubic centimeter in washing solutions*

Time	Temperature	Hydrochloric acid		Sodium carbonate-trisodium phosphate	
		Bottle 1	Bottle 2	Bottle 1	Bottle 2
11:30 a.m.	110° F.	258,000	468,000	67,500	70,600
11:00 p.m.	72° F.	30,000	18,000	3,600	200
Percentage of kill		93.3		97.4	

Representative plates from the series presented by table 7, showing the number of viable blue-mold spores in the washing solutions before and after the 11½-hour stand, are shown in figure 1.

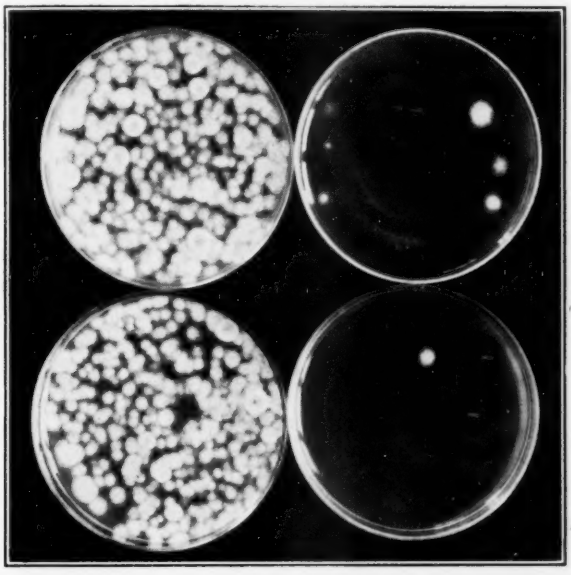

FIG. 1. Cultures from 1 per cent hydrochloric acid cleaner before and after the 11½-hour stand at a temperature gradually lowering from 110° F. to 72° F., showing the decrease in the number of blue-mold colonies.

In this experiment the time and temperature factors were taken at conservative levels. The time occupied by the fall of the temperature and its restoration to the degree used in cleaning is usually 13 hours; in this experiment it was lowered for 11½ hours. In commercial operations the temperature drops to 70–85° F. by 4:30 a. m. and rises to 100–110° F. by 7:00 a. m.; this rise was omitted.

It is believed that the blue-mold spores will nearly all be killed during the overnight stand under the present practice of heating the washing solutions and that formaldehyde treatments, the daily changing of solutions, or other measures aiming at reduction of spore load are unnecessary. It should be noted that this test shows a close correlation of the effect of commercial solutions and the laboratory-test solutions as presented in the following experiments.

Hydrochloric Acid Cleaner. The effect of hydrochloric acid on blue-mold spores at the maximum strength of commercial cleaning and at various temperatures was determined (Table 8). The 2 series at room temperature

TABLE 8.—*Number of viable spores of blue mold per cubic centimeter obtained at intervals from spore suspensions in 3 per cent HCl at different temperatures*

Time	Water check	3% commercial acid		
		Flask 1	Flask 2	Flask 3
Room temperature				
First series				
0 hours	218	192	211	224
6 " later	297	312	289	276
12 " "	226	127	225	212
24 " "	306	201	196	227
36 " "	282	96	102	127
48 " "	235	16	14	32
Second series				
0 hours	1,370	1,143	1,696	1,496
6 " later	1,706	2,023	1,773	1,973
12 " "	1,050	1,333	1,770	1,510
24 " "	1,408	1,073	1,046	1,143
36 " "	1,243	640	510	536
48 " "	1,506	226	163	166
90° F. temperature				
0 hours	4,120	4,163	4,500	4,036
1 hour later	3,920	4,486	4,433	4,043
6 hours "	3,816	3,063	2,916	3,086
12 " "	4,053	1,726	1,793	1,736
24 " "	4,220	393	266	130
48 " "	4,396	10	20	10
72 " "	4,330	0	0	0
100° F. temperature				
0 hours	2,653	2,080	2,713	2,263
1 hour later	2,343	2,663	2,900	1,970
6 hours "	1,990	73	40	16
12 " "	1,893	0	0	0
24 " "	3	0	0	0
48 " "	0	0	0	0
72 " "	0	0	0	0
110° F. temperature				
0 hours	4,190	5,290	5,720	4,830
1 hour later	2,866	1,996	2,970	1,886
2 hours "	1,870	143	1,666	596
3 " "	2,083	10	750	26
4 " "	1,883	0	6	0
5 " "	1,503	0	0	0
6 " "	1,253	0	0	0
7 " "	1,370	0	0	0
8 " "	1,190	0	0	0
10 " "	13	0	0	0
12 " "	0	0	0	0
24 " "	0	0	0	0
48 " "	0	0	0	0
72 " "	0	0	0	0

are identical, except for the greater spore concentration in the second. In the flasks with heavy spore loads an average of 12.8 per cent of the spores were viable at the end of 48 hours' exposure as compared to 9.5 per cent in the flasks with the lower concentrations. This is in agreement with the findings of Henderson-Smith (6) and is also observed in the other series to a lesser degree. As it was felt that the variations caused by the factor of unequal spore loads were slight, extreme concentrations of spores were used to lessen error in counting.

Commercial HCl exerted a marked toxic effect in 48 hours at room temperature as compared to water checks, which showed no reduction in numbers. The spores were all killed in the acid solution in 72 hours at 90° F. as contrasted to the water check in which none were killed. The spores were all killed in 12 hours at 100° F. as compared to 48 hours for the water check. The spores were all killed in 5 hours at 110° F. in the acid solution as compared to 12 hours for the water check.

Sodium Carbonate-Trisodium Phosphate Cleaner. The effect of this cleaner on blue-mold spores was tested in the same manner as was the acid solution (Table 9).

The sodium carbonate-trisodium phosphate cleaner exerted a marked toxic effect in 72 hours at room temperature as compared to water checks, which showed no killing. The spores were all killed in 24 hours at 90° F. and the water check was not reduced in 72 hours. In flasks held at 100° F. the spores were all killed in 12 hours as contrasted to 48 hours required by the water check. Spores were all killed in 7 hours at 110° F. as contrasted to a few remaining viable in the water check after 12 hours.

Sodium Carbonate-Borax Cleaner. Difficulty in testing this cleaner was encountered at first, due to its growth-inhibiting properties when transferred from the 1 to 10-dilution tube to the poured plate. Colony formation was irregular in time of appearance and in size as contrasted to water checks.

The use of medium of pH 4.5 gave the same results as ordinary medium of pH 6.2 in tests with the cleaner, with water checks, and with the sodium carbonate and borax separately in strengths at which they occurred in the cleaner. It was found that the borax constituent at strengths used in the cleaner exerted little toxic or inhibiting action, the effect of the cleaner being due to its content of sodium carbonate. The inhibiting effect of the cleaner was due to some property of the sodium carbonate not altered by reduction of its active alkalinity.

The technique used in testing this cleaner was correspondingly altered to reduce this inhibiting action. A flask containing 300 cc. sterile distilled

TABLE 9.—*Number of viable spores of blue mold per cubic centimeter obtained at intervals from spore suspensions in sodium carbonate-trisodium phosphate cleaner, 60 lbs. per 100 gal., at different temperatures*

Time	Water check	Sodium carbonate-trisodium phosphate		
		Flask 1	Flask 2	Flask 3
Room temperature				
0 hours	3,416	4,523	3,253	3,580
1 hour later	3,166	3,450	3,526	3,430
6 hours "	3,250	2,960	2,980	2,940
12 " "	3,003	2,610	2,380	2,263
25 " "	3,146	1,996	1,973	1,770
36 " "	3,133	1,310	1,226	1,073
48 " "	3,206	596	526	30
72 " "	3,170	260	60	223
90° F. temperature				
0 hours	3,983	3,766	2,866	3,803
1 hour later	3,966	3,250	3,203	3,153
6 hours "	4,580	1,866	1,016	1,393
12 " "	4,016	26	320	46
24 " "	4,153	0	0	0
36 " "	3,966	0	0	0
48 " "	3,703	0	0	0
72 " "	4,230	0	0	0
100° F. temperature				
0 hours	3,396	3,450	3,686	3,040
1 hour later	3,423	2,273	2,060	1,743
6 hours "	2,380	3	10	70
12 " "	2,390	0	0	0
24 " "	636	0	0	0
36 " "	6	0	0	0
48 " "	0	0	0	0
72 " "	0	0	0	0
110° F. temperature				
0 hours	3,890	3,483	3,953	3,850
1 hour later	2,676	2,533	2,623	3,586
2 hours "	976	213	60	1,846
3 " "	606	30	0	600
4 " "	520	10	6	150
5 " "	380	10	3	16
6 " "	530	0	0	3
7 " "	286	0	0	0
8 " "	126	0	0	0
10 " "	20	0	0	0
12 " "	1	0	0	0

TABLE 10.—*Number of viable spores of blue mold per cubic centimeter obtained at intervals from spore suspensions in sodium carbonate-borax cleaner, 1½ lbs. per gal., at different temperatures*

Time	Water check	Sodium carbonate-borax		
		Flask 1	Flask 2	Flask 3
Room temperature				
0 hours	48,100	40,900	44,100	41,700
12 " later	53,800	12,400	14,900	12,600
24 " "	49,100	13,000	8,400	9,200
36 " "	41,600	7,800	14,300	19,700
48 " "	46,800	3,800	6,600	5,100
60 " "	46,600	4,600	3,200	2,800
72 " "	48,500	1,200	1,400	1,700
84 " "	43,800	800	1,600	1,100
96 " "	52,900	100	300	500
90° F. temperature				
0 hours	42,400	30,200	30,900	41,000
12 " later	46,500	29,700	26,200	27,900
24 " "	37,000	23,800	25,300	24,600
36 " "	39,900	14,900	13,600	15,800
48 " "	43,100	7,300	7,200	6,200
60 " "	33,900	4,500	3,000	2,800
72 " "	37,800	2,200	1,500	0
84 " "	44,200	0	0	0
96 " "	45,500	0	0	0
100° F. temperature				
0 hours	31,400	33,200	32,900	34,000
12 " later	22,200	22,600	21,700	25,500
24 " "	4,500	7,800	2,600	6,400
30 " "	900	1,300	2,100	2,000
36 " "	0	700	300	600
48 " "	300	100	0	0
60 " "	100	0	0	0
110° F. temperature				
0 hours	44,700	24,100	25,400	29,400
1 hour later	25,700	26,000	22,600	23,400
2 hours "	26,400	21,900	20,200	19,600
3 " "	29,200	19,700	18,700	24,500
4 " "	27,300	17,800	19,300	23,700
5 " "	21,900	11,600	15,600	17,700
6 " "	16,900	9,500	14,000	16,100
7 " "	36,000	6,100	7,400	11,800
11 " "	100	600	1,100	1,500

water was used instead of a 9-cc. water blank, and 1 cc. of the test solution was transferred to it. After being shaken 5 minutes, 1 cc. of this 1 to 300 dilution was transferred to a Petri dish and 2 per cent dextrose potato-agar poured over it. Greater spore concentrations were used than in other series due to the greater dilution employed. In 10 plates made from each of 3 flasks treated in the above manner there was no more variation than from a similar series run from a water check. Table 10 shows the action of this cleaner on spores of blue mold.

This cleaner exerted a slight toxic effect in 48 hours at room temperature and a considerable toxic action in 96 hours as compared to the water check, in which the spores were not killed in the longer period. The spores were all killed in 84 hours at 90° F. as contrasted to no reduction in the water check in 96 hours. In flasks held at 100° F. the spores were killed in 60 hours, nearly the same as in the water check. The number of viable spores was considerably reduced in 11 hours at 110° F., nearly the same as in the water check.

THE EFFECT OF SODIUM HYPOCHLORITE ON BLUE-MOLD SPORES

A great deal of the spore contamination of the washing tank is obtained from the air of the packing house and from the washing of the fruit. Dirty picking boxes that have enormous loads of blue-mold spores are frequently used. Huber[8] found from 127,860 (5 per cent blue mold, equivalent to 6,393 spores) to 531,200 colonies (30 per cent blue mold, equivalent to 159,360 spores) per square inch on the inside surfaces of the bottom boards of dirty boxes. These spores scatter onto the fruit and hence get into the washing tank or are blown into the air and settle on equipment. It has been found that the condensed steam from the heated washing solutions would enable blue mold to grow on the near-by overhead packing-house timbers, forming striking incrustations. Spores from these sources will increase the spore load of the washing tank directly or indirectly and will settle on fruit after washing. As this will increase the percentage of decay, reduction of these sources of contamination is desirable.

Yesair (13) found that *Penicillium expansum* was one of the most common molds on the walls and equipment of meat-packing houses. He used sodium hypochlorite solutions of different strengths for 5- to 10-minute periods successfully in reducing spore load of these places.

A commercial sodium hypochlorite solution[9] containing 3.4 per cent available chlorine was tested for its effect on the viability of blue-mold spores.

[8] Huber, G. A. Fungous flora of the normal apple: the sources of contamination and spore load. Unpubl. thesis, Washington State College, 1931.

[9] Chlorosan (now called Westochlor), furnished by the West Disinfecting Co., Seattle, Washington.

In 6 trials all of the spores in a concentrated suspension in a solution of 3 oz. of this compound per gallon were killed in 15 minutes and at a strength of 0.1 oz. per gallon in 3 hours.

Blocks 1 in. square were cut from apple-box sides and dipped in a suspension of blue-mold spores. Washings were made from some of these by the method described by Huber (7) for determining the spore load of apples and the average spore load determined by plating out portions of the wash water. Other blocks were sprayed with the commercial sodium hypochlorite solution (3 oz. per gallon) and washings made to determine the load of viable spores. Blocks averaged 118,400 viable spores per square inch after dipping in the suspension. Four and a half hours after being sprayed with the sodium hypochlorite solution other blocks had an average of 12,700 viable spores per square inch, or about a 90 per cent kill. As the boxes from which these blocks were taken were slightly dirty some free organic matter was present. Thus, spraying with sodium hypochlorite solutions would be an easy and reasonably effective way of reducing the spore load of packing-house equipment.

DISCUSSION

The practice of washing apples for removal of arsenical residue is now well established in the principal apple districts. Hydrochloric acid cleaner is the one principally used, but alkaline washes containing considerable amounts of sodium carbonate are also widely employed. It has been repeatedly shown that the washing tank is one of the main sources of the spore load of apples. At present these solutions are used without special consideration of decay prevention because no solutions have been found that are of practical value.

It has been shown in this paper that, contrary to the previous reports on Penicillium species, temperature of the solution within the ranges employed does exert a lethal effect in itself on spores of *Penicillium expansum*. This action was markedly increased by addition of the washing compound. The action of both temperature and cleaners is summarized in table 11.

The lethal action of the sodium carbonate-borax cleaner was shown to be due to the constituent sodium carbonate, and the borax in the amounts used was only slightly toxic.

It has been stated by other investigators that the washing solutions are not sufficiently toxic to blue-mold spores to be of any value in reducing the load of viable spores under the conditions used. It has also been stated that the temperature factor is unimportant. In this paper it has been shown that the temperature and the toxicity of the cleaning solution are important factors in the lethal action of cleaning solutions. The action is of a slow type, and no action would occur on spores on apples during their

TABLE 11.—*Summary of the effect of cleaning solutions and of water at different temperatures on blue-mold spores*

Temperature	Water check	Washing solution		
		Hydrochloric acid	Sodium carbonate-trisodium phosphate	Sodium carbonate-borax
Room	None killed in 96 hours	87–90% killed in 48 hours	89% killed in 48 hours 95% killed in 72 hours	87% killed in 48 hours; 90% killed in 72 hours; 97% killed in 96 hours
90° F.	None killed in 96 hours	All killed in 72 hours	All killed in 24 hours	All killed in 72–84 hours
100° F.	All killed in 48 hours	All killed in 12 hours	All killed in 12 hours	All killed in 48–60 hours
110° F.	All killed in 12 hours	All killed in 4–5 hours	All killed in 6–7 hours	86% killed in 11 hours

brief exposure in washing. For that reason the claims of the manufacturers that their cleaners will effectively control blue mold are unjustified. The cleaners as used at present, however, will keep the tanks reduced to a concentration of viable spores whose maximum will be that accumulated in a day's run.

Tests with samples taken from washing solutions at the end of a day's run showed a 93.3 per cent and 97.4 per cent kill of the spores in acid and sodium carbonate-trisodium phosphate cleaners, respectively, after 11½ hours' exposure at a temperature gradually decreasing from 110° to 72° F. It is not probable that any special means of reduction of spore load of the washing tanks from day to day is needed at present.

A close correlation existed between the laboratory tests and the conditions actually occurring in the washing tanks.

The lethal action on young and old spores was shown not to vary so markedly with their age in the case of high temperatures as with the toxicity of cleaners. The action of hydrochloric acid and sodium hypochlorite solution was shown to be inversely related to spore age.

The lethal effect of the cleaners was generally found to vary inversely with the spore concentration when other conditions were equal. The variations was so slight, however, that it is felt that the number of spores is not a significant factor in their resistance to either temperature or toxic agents in packing-house operations.

It was found that spores germinated in 7 days at 32° F. in 25, 50, and 100 per cent apple juice and in Brown's synthetic medium. Spores frozen in 10 per cent apple juice and held at 21°–25° F. for 4 days germinated readily on being returned to room temperature. It is probable that infection of apples can occur at 32° F. from spores germinating in storage and that infection has not always occurred previous to the drop in temperature to 32° F.

Sodium hypochlorite solutions have been found to have high toxicity for *Penicillium expansum* spores and may be used as a spray in packing houses to reduce spore contamination. The effect of these solutions when applied directly to the fruit is being investigated.

SUMMARY

1. The washing tank is one of the main sources of contamination of apples with blue mold, and the reduction of its load of viable spores directly or indirectly is desirable.

2. Age is of less importance in resistance of *Penicillium expansum* spores to high temperatures than to toxic chemicals. Old spores were consistently more resistant to the toxic agents tested than young ones.

3. Spore concentration in washing solutions is not a significant factor in resistance of the spores to either the temperature factor or to toxic agents in washing tanks.

4. The toxic action of the principal cleaning solutions now used is of no greater importance than that of the temperatures at which they are used, although the addition of these to the water decreases the time required for a complete kill.

5. The toxicity of the sodium carbonate-borax cleaner is due to the constituent sodium carbonate, and this cleaner has marked inhibitory action in high dilution.

6. Practically all of the blue-mold spores contained in the cleaning solutions are killed during the overnight stand, though the tanks are not being heated during that time.

7. Special means of reducing the spore load of the washing tanks from day to day are unnecessary under present conditions of operation.

8. Blue-mold spores failed to germinate at room temperature or 90° F. in sterile distilled or tap water. They germinated in 12 hours in 2 per cent apple-juice solution at room temperature but did not in 14 days at 90° F.

9. Germination of blue-mold spores occurred in sterile apple juice at 32° F. in 7 days.

10. Spores held at 21°–25° F. in 10 per cent apple juice for 101 hours germinated readily on being returned to room temperature.

11. Sodium hypochlorite solutions are very toxic to *P. expansum* spores and could be used to advantage for spraying packing-house equipment to reduce spore contamination.

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A PRELIMINARY STUDY ON THE USE OF INDELIBLE PENCILS AS INDICATORS OF THE MOISTURE CONTENT OF WOOD

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When an indelible (anilin) pencil is drawn across a moist or wet surface the mark is of a very different color from that made on a dry surface. The common purple pencils of this type, for example, leave a somewhat glossy pale gray mark on a dry surface, but a dull and more deeply colored purple mark on a wet surface. This generally known property of such pencils suggested a series of studies looking toward the correlation of the streak color with the moisture content of wood. These studies were conducted at the School of Forestry of the University of Idaho during the academic year of 1928–29.

Following inquiries, a number of pencil manufacturers submitted for testing sample pencils of various colors. Each of these pencils was given an arbitrary number for convenience in keeping records. The 4 used principally in the investigation are listed in table 1.

TABLE 1.—*List of principal indelible pencils used, with maker's name and designation, and colors of streaks*

Pencil No.	Maker	Maker's designation	Color of streak			
			On pale wood		On white writing paper	
			Dry	Wetted and dried	Dry	Wetted and dried
8	Joseph Dixon Crucible Co.	Eldorado, master copying, 213 soft	Neutral gray	Blackish violet	Violet gray	Dark violet
13	American Lead Pencil Co.	A special indelible	Rose doree	Begonia rose	Carmine	Begonia rose
15	Koh-i-noor Pencil Co.	Mephisto copying, 73F.	Neuvider green	Vivid green	Dark viridian green	Vivid green
20	Blaisdell Pencil Co.	Super-thin, 754	Light orange yellow	Light cadmium	Deep chrome	Strontian yellow

The color streaks of these 4 pencils can be described in general terms as purple, red, green, and yellow, respectively. More precise designations for the streaks on pale-colored wood (sapwood of western white pine (*Pinus monticola* D. Don.)) and on white writing paper are given in the last 4 columns of table 1. These are based on comparisons with the plates in

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R. Ridgway's "Color Standards and Color Nomenclature" (1912). For the "wet" color in the case of the wood the surface was first moistened by sponging, while on the paper patches of the dry color were wetted with a sponge.

In the earlier stages of the experiments it was thought that a set of pencils might be found that would show marked color changes due to solution of the coloring material and corresponding to diverse moisture contents of the wood, and the several pencils were tested with this in mind. Later, when considerable preliminary work had been disposed of, a systematic test was made with about 100 blocks of western white pine. In this series 48 different moisture contents were represented, ranging from 16 per cent to 87 per cent, on the oven-dry basis. The 4 pencils above listed were tested on each block. When the test blocks, after oven drying, were arranged in order of their moisture content prior to drying it became evident that solution of the streak could not be clearly correlated with any definite moisture content of the test material. On the contrary, there was for each pencil a whole series of streaks of slightly different color tone referable to small differences in moisture content of the test blocks. The differences in tone between any 2 adjacent blocks were scarcely distinguishable, although the differences between the streaks on blocks containing, respectively, 19 and 69 per cent moisture, for instance, were quite pronounced.

After this, these studies were directed toward preparing sets of standards with the several pencils and with various species of wood and testing out the utility of such standards by actual trial. Most of the work was done with streaks of the 4 pencils listed in table 1 on blocks of western-white-pine sapwood, and the following discourse has to do primarily with standards and test blocks of this material. The results from tests with other pencils and other wood species are not numerous enough to warrant detailed presentation.

In the main, small blocks about 1.3 cm. square and 2.5 cm. long were used in these studies. These blocks were placed 10 in a Stender dish and an amount of water was added sufficient to give a calculated average moisture content. This amount was based on the air-dry weight of the group of blocks and the moisture content, ascertained by drying, for sample blocks from the same stick. Sometimes an unmeasured amount of water was added. The Stender dishes were about 37 mm. high and 76 mm. in diameter, inside, and the covers had a ground groove fitting tightly against the ground edge of the dish. Generally, the dishes were left standing a day after adding the water and covering. They were then subjected in an autoclave to a temperature of 98° C. for an hour and finally left standing another day before the tests were made. It was found that this treatment resulted in very uniform distribution of the moisture.

In making a test the block was first weighed and then split open and streaks quickly made from the center to the end, 2 streaks for each pencil (using different sides of the lead), 2 pencils to each freshly exposed surface. An effort was made to use uniform pressure in making these streaks. After drawing the streaks the pencil points were dried by rubbing on a piece of paper. The blocks were oven-dried and the moisture content at the time of making the streak computed. These blocks, suitably mounted on cards, then served as standards in subsequent studies.

With such standards, some 300 estimates were made of the moisture content of blocks and the actual moisture content was then computed. Some results of such tests will suggest the possibilities of the pencil-streak method.

Of 82 estimates made with standard streaks of pencil No. 8, 52 per cent were within 5 points (above or below) the actual moisture content in percentage, 72 per cent within 10 points. The following figures give results for the 4 pencils here considered:

Pencil No.	Correct estimates	Estimates within 5 points	Estimates within 10 points	Total tests
	Per ct.	*Per ct.*	*Per ct.*	
8	5	52	72	82
13	5	54	71	82
15	6	58	74	81
20	7	57	74	81

While these percentages can not be considered acceptably close for all types of work requiring a knowledge of moisture content, yet they might, for example, prove to be close enough in brush-burning work. At any rate, they do suggest the possibilities of the method. At the present stage of the study it can not be stated with assurance just why the errors should be so large. In some cases they are perhaps attributable to an insufficient set of standards. And undoubtedly the matter of practice is to be considered an important factor. Possibly streaks of some other pencil than the 4 studied most intensively, namely, Nos. 8, 13, 15, and 20, would give more satisfactory standards than these. The standards used were kept in covered cardboard boxes after drying, except for the short time during which comparisons were being made, and exposure to direct sunlight was always avoided.

The method thus far developed by these tests gives promise of utility in certain ways, especially where it is desirable to study the distribution of moisture in wood with pronounced differences in moisture content. An example will serve to illustrate some of the possibilities of this method.

An air-dry block of western-white-pine heartwood 20 cm. high and 5 cm. thick was kept standing on end in water 3 or 4 cm. deep, the water being brought up to the original level from time to time. At the end of 7½ weeks, the outside was tested with 3 indelible pencils, and it was found that at about the level of the water surface, 4.5 cm., and abrupt color change took place from dry colors (corresponding to less than 16 per cent moisture content) to wet colors (corresponding to more than 87 per cent moisture content). On a freshly split surface roughly parallel to the outside surface and 1 cm. from it, the change occurred 2.5 cm. from the bottom. That is to say, in 7½ weeks with the water in which it was immersed usually at least 3 cm. deep the penetration inside was to a lesser height than the depth of the water. On the outside of the block the moisture had risen slightly above the water level.

After 3 months the water was allowed to evaporate without being replenished. About 2 or 3 weeks after the water was gone, pencil tests were again made. On the outside at this time and 1 cm. from the edge the moisture content, as shown by the streak colors, was less than 16 per cent. On a face 2 cm. from the edge there was a pronounced moist area about 3 cm. long and 2.5 cm. wide. Outside of this area, the moisture content was apparently less than 16 per cent. The transition from dry to wet wood was rather abrupt, the moisture content of the outer portion of the moist region being apparently at least 70 per cent. At the center the moisture content was probably over 90 per cent. The moist area extended to the bottom of the block and was situated in the middle of the section.

E. E. Hubert, of the Idaho School of Forestry, observed a marked color change occurring at the point where the moisture content of a block began to inhibit the growth of a blue-stain fungus. At this time, no standards were available for interpretation of the percentage moisture contents.

On the whole, it seems likely that this method, when perfected, would in some cases give more satisfactory information about the moisture in a piece of wood than that obtainable from the ordinary method of weighing and oven drying. In this latter method the *average* moisture content of the test piece is ascertained. If, for instance, a disk be cut from a stick in a brush pile the average moisture content of this can readily be ascertained. A more nearly exact picture would, of course, be given by making separate tests of blocks from the upper and lower parts of the stick as it lay in the pile. But, if the moisture distribution could be ascertained by making pencil streaks on freshly exposed surfaces of such wood, we ought to obtain thereby a far better understanding of the moisture conditions in such a stick. It seems likely that the method could be used also in investigating in detail moisture conditions in various parts of a pile. Such precise

information might very well prove of the highest significance in interpreting the activities of fungi or insects in brush-pile wood and in laying plans for brush-burning activities. A further application that may be envisioned is in forest-fire studies, where it is desirable to ascertain, as a measure of inflammability, the moisture content in various parts of logs and branches lying on the ground.

Possibly the pencil method could also be substituted for the oven-drying method in making studies of the moisture content of test material in which the moisture distribution is essentially uniform. It is, however, to be kept in mind that there are still several problems to be solved in connection with the method here discussed before it can be used except upon rather a limited scale by expert investigators. Some of these problems will now be briefly discussed.

In the first place, it seems unquestionable that the color tone will vary with the color of the wood upon which the streak is drawn. It is possible, too, that the tone may vary between streaks made on wood of the same color but of different species. It may be that the color will not be constant for pencils from different batches of the same brand and style number.

Another problem has to do with the question of permanence of standards. A few preliminary tests were made to ascertain the effect of strong sunlight upon the colors. For this purpose a series of test blocks was shellacked and mounted on a board, "Superior Pure Shellac, White," manufactured by the Glidden Company (of Cleveland) being used. These blocks (of western-white-pine sapwood) had been oven dried after marking with pencils Nos. 8, 13, 15, and 20, and the moisture content ascertained. One half of each streak on the test blocks was covered with black paper. They were then placed in a south window to receive full sunlight and left for a few days.

Examination of these test blocks disclosed that in most cases the colors had completely faded out. At 6 per cent moisture content and also at 84, 90, 99, 105, 119, and 125 per cent in the case of No. 15, part of the streak was still discernible, but the color was much paler than on the part of the block that had been shaded. At 6 per cent in the case of No. 20 the streak was orange rather than yellow. In the case of No. 8 streaks, the color was not appreciably faded at 6 and at 15 per cent. The other streaks had changed from purple to gray (black at 125 per cent), but graduated differences in intensity were still obvious.

On the whole, the study of this phase of the subject indicates that the streaks of Nos. 13, 15, and 20 have no permanency when exposed to bright sunlight under the conditions of the test, and the streaks of No. 8 are modified to such an extent that they would be of doubtful value as standards.

Another test was directed toward ascertaining the possibility of waterproofing color standards with shellac, so that they could be handled without risk of alteration due to accidental access of moisture. Streaks were made on dry sapwood of western white pine and these then shellacked. Streaks of some of the pencils were found to run notably, and the color of the streak was appreciably altered by the shellacking in a considerable number of cases.

It is to be noted that there was a certain amount of color change, also, incident to baking in oven drying to ascertain the moisture content for use as a standard.

All things considered, it would be preferable not to use the pencil streaks themselves for standards but to have permanent, durable standards prepared to match the streak color corresponding to each percentage index (and for each wood species and test pencil it is desired to use). It is also to be remembered that any system depending upon the matching of colors is somewhat restricted in availability because of the inadequacy of certain artificial lights for the purpose and because of the difference in ability of different persons to distinguish closely allied hues, shades, and tints. Perhaps the pencil manufacturers would be interested in developing such standards.

SUMMARY

This paper describes some preliminary tests on the use of indelible pencil streaks for ascertaining the moisture content of wood. This method apparently offers a promising means of studying the moisture distribution in a test sample of wood, at least when pronounced differences in moisture content are found in different parts of the test piece. For investigating the moisture in wood where the distribution is irregular, as in a stick from a brush pile or a fallen log, it seems likely that this method, when perfected and in the hands of a skilled technician, would give more satisfactory information than that obtainable by the ordinary methods of weighing and oven drying, which, of course, give only the *average* moisture content of the test piece. Possibly the pencil method could also be substituted for the oven-drying method in making studies of the moisture content of test material in which the moisture distribution is essentially uniform. These studies have, however, not yet reached a stage where this seems feasible.

A number of problems connected with this method are still to be solved. These have to do mainly with the preparation of usable permanent standards.

CARMEL, CALIF.

MILD STREAK OF BLACK RASPBERRIES[1]

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The eastern blue-stem or streak virous disease of black raspberries was first described by Wilcox in 1923 (6) from Cuyahoga County, Ohio. Since then, for this State as a whole, streak has been found to be the most prevalent of the virous diseases of black raspberries. In Ohio, the symptoms have usually been distinct, typical, and uniform and have agreed closely with Wilcox's original description.

Following some field experiences with a peculiar streak type of disease found in a few black raspberry plantings in northeastern Ohio in 1927 and 1928, the writer decided that there were two separate kinds of streak in black raspberries. Subsequent observations and studies, made each season since then, have tended to confirm this belief. The name "mild streak" was given to the new type. The older and better-known form was designated "severe streak." These names seemed descriptive of the differences in the effects produced on host plants by the two forms. In such a nomenclature, the name severe streak is synonymous with eastern blue-stem or streak as described originally by Wilcox.

This conception of two separate, but quite similar, diseases seems to explain the confusion evident in the literature as to the exact symptoms of streak and their constancy and the identification of the disease in different berry-growing districts in eastern United States. Bennett (1) found that the mottling of foliage and the streaking of canes tended to be indistinct under Michigan conditions. In 1927, Rankin (4, p. 48) described a disease type in black raspberries in New York that showed some of the symptoms of streak, but "the blue marks on the canes described for streak rarely showed and the progressive later stages for streak were not evident." It now seems probable that these investigators were working, for the most part, with the mild type of streak. In a recent classification of raspberry viruses, Rankin (5) adopted the conception presented here, listed mild streak as a separate virous disease, and gave a brief description of its symptoms.

Mild streak is a disease of cultivated varieties of the black raspberry, *Rubus occidentalis* L., and is believed to be caused by a virus. The malady has not been recognized on any other host.

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SYMPTOMS

Black-raspberry plants affected with mild streak are never stunted seriously; if heavily fertilized, they may make remarkable growth.

The most constant foliage symptom exhibited by a diseased plant is the characteristic hooking, recurving, and twisting of the midribs of leaflets at the tips of the new canes (turions). Usually, the midribs of all other leaves also curl slightly and arch downward, giving the entire plant a drooped or faintly "rosetted" appearance. The foliage, in general, also displays a green color just a shade darker than normal. The old leaves are never blotched or mottled, although they may develop a faint lightening of color along the main veins.

Turions may or may not be streaked at their bases. The longitudinal streaks, if present, are narrow (½ mm. or less in width). They are steel-blue and gray in color (approximating Hathi gray of Ridgway's color chart) and are obscured by the translucent epidermis and cuticle. Such streaks may be present also on the stems of fruiting laterals, on petioles, and on flower or fruit pedicels.

The most distinctive and diagnostic single symptom of mild streak is the manner in which the fruit is affected. The drupelets on individual berries develop unevenly. This effect can be noted from the time of flowering until the berries are gone. When the normal time for the ripening of the fruits is reached, on the same berry some drupelets will be hard and unripe (some red, others green), a few will be ripe but of disagreeable flavor, and still others will have ripened prematurely and decayed. As a consequence, the berries on mild streak plants are "piebald," small, seedy, and distasteful. A black-raspberry plant with mild streak may live for several years without a marked reduction in vigor and yet never bear a crop of marketable fruit. Because of this particular affection of the fruit, the mild form of streak occasions more direct economic loss per plant infected than does severe streak.

Symptoms of mild streak are expressed most noticeably during that period of the year when temperatures are highest. In Ohio, the disease may be distinguished clearly in July and August. In some seasons the symptoms are more pronounced than others, correlating with the general temperature range. Plants that are given good culture and are growing rapidly express symptoms (other than stunting) more markedly than neglected, slow-growing ones. Although the phenomenon has not yet been witnessed by the writer, it is considered possible that the mild-streak virus may be resident in a black-raspberry plant and remain completely masked throughout an entire season.

COMPARISON OF MILD STREAK AND SEVERE STREAK

Several of the symptoms of mild streak indicate a close relationship of this disease with severe streak. The same typical recurving and hooking of the midribs of tip leaflets of the turions is a symptom common to both diseases and, in both, it is identical in appearance and extent. A general rosetted appearance of affected plants is noticeable with both diseases; but, since the growth is not stunted much by mild streak, the rosetting is less pronounced. Streaks occurring on turion bases, on stems of fruiting laterals, and on petioles are a common symptom, but the streaks themselves are dissimilar.

The two diseases coincide in their seasons of symptom expression and in their temperature responses. Both diseases seem rather specific to black raspberry, though Wilcox (6) cites instances of severe streak on blackberry plants.

Despite these similarities, mild streak may be distinguished from severe streak rather readily by certain symptomatic differences. In the writer's experience in Ohio, a conspicuous and distinctive blotching or gross mottling of the older foliage is present in all fully developed cases of severe streak; this character is absent or very faint in the mild type. The streaks, from which both diseases derive their names, are different: broad, short, and dark blue or brown in the severe; slender, long, light blue-gray in the mild. The differences in host growth are marked: severe streak causes an appreciable and comparatively rapid stunting by shortening the internodes, the final result being a decided depression of longitudinal growth; mild streak produces only a slight stunting, and, under circumstances favorable for host growth, this is not appreciable. The stunting produced by severe streak becomes more severe with each successive season; the slight stunting of mild streak does not seem to be cumulative. The peculiar affection of fruits by mild streak has been described above. In contrast, in the case of severe streak the drupelets of the berries develop uniformly, and, though the fruits are of poor quality, they are marketable.

Mild streak is not likely to be confused with any other of the virous diseases of raspberry. However, leaf hoppers sometimes produce foliage injury deceivingly like that of mild streak. If fruits are present on suspected plants, an examination of them will reveal mild streak. Leaflets that have been attacked by hoppers show an injured brown area on the dorsal surface of their midribs; this usually serves to distinguish hopper injury.

ETIOLOGY

The belief that mild streak is a virous disease is based on field evidence. No pathogenic agency that conceivably could cause the disease has ever been

found associated with diseased individuals, and in no case has it been possible to correlate the occurrence of diseased plants with any environmental factor.

Symptoms are expressed by all plant parts aboveground; this would indicate the systemic nature of the disease. The symptoms themselves, especially in their mild resemblance to those of severe streak, are suggestive of a virus, and, as stated above, a direct correlation exists between symptom expression and temperature.

Mild streak recurs in succeeding seasons on plants once affected but does not develop into the severe type. The disease is disseminated by the vegetative propagation of diseased individuals.

Under most conditions, the rate of spread of mild streak is slow, about doubling each season in the plantings observed. But, in vigorous plantings, spread has been seen to take place much more rapidly. Plants adjacent to a diseased individual are apt to become infected, but the disease seemingly may be carried almost as readily to more distant plants. Spread very commonly takes place to the leeward of an infection source. The distribution and rate and time of spread are not only indicative of a virous trouble but also suggestive of an insect vector.

The successful experimental transmission of streak, either mild or severe, has not yet been accomplished. Consequently, evidence of infection of the virous nature of mild streak is lacking. Nor has it been possible to test for separate viruses by inoculation work.

DISTRIBUTION

No specific survey for mild streak has been made in Ohio, but the disease has been found definitely in 12 plantings on 8 different farms, in 5 counties. Of the 12 plantings, 11 were of the Cumberland variety; the other one, Plum Farmer. In 7 of the 12 plantings, severe streak was present also, furnishing opportunities for critical comparisons of symptoms. Six of the 8 farms where the disease has been found were located in the easternmost tier of counties (Columbiana, Mahoning, and Ashtabula). One heavily infected planting was in Cuyahoga County and another in Sandusky County. On the whole, the occurrence of mild streak in Ohio is rather rare in comparison with the severe type.

Bennett (2, p. 16) found streak to be "less common in Michigan [than in Ohio] and as yet [1928] it has not spread very rapidly or widely." He remarked in conversation with the writer that the mild type was much more frequent in Michigan than was the severe one.

"Mild streak is more or less prevalent in central and western New York" and is much more common there than severe streak (Rankin, 5, p. 9).

Judging from the descriptions of symptoms given, Berkeley (3) has reported what seems to be mild streak of black raspberries occurring in two plantings in Ontario, Canada.

VARIETAL SUSCEPTIBILITY

In Ohio and Michigan, the black-raspberry variety Cumberland has been the one most frequently affected with mild streak. One instance in Plum Farmer has been found in Ohio. At Rogers, Ohio, an extensive planting of the variety Improved Kansas has been growing for several years beside a large Cumberland planting that contains numerous individuals affected with mild streak. During the 3 years these plantings have been under observation, the mild streak has spread rather widely within the Cumberland planting, whereas not one of the adjacent Improved Kansas plants has become infected.

In New York the variety Cumberland is grown but little. There, Rankin (5) has reported that mild streak is particularly prevalent in the varieties Ohio and Kansas.

From these rather meager observations it would seem that the relative susceptibility of black-raspberry varieties to mild streak probably corresponds closely with that given by Wilcox (6) for severe streak, and it seems equally true in the case of mild streak that susceptibility is correlated closely with varietal characteristics of growth habit and vigor—the more vigorously growing varieties seeming more susceptible.

SUMMARY

Mild streak is a disease of the black raspberry, *Rubus occidentalis* L., believed to be caused by a virus. The malady has not been recognized on any other host.

A description of symptoms is given. Symptom expression has been found to be most pronounced during the hot summer months.

The belief that mild streak is a virous disease is based on field evidence. The successful experimental transmission of mild streak has not yet been accomplished and final experimental evidence of infection is lacking.

The distribution, rate, and time of spread of mild streak are not only indicative of a virous trouble but also suggestive of an insect vector.

The names "mild streak" and "severe streak" are suggested to differentiate between what seem to be two separate diseases of similar nature. In this concept, severe streak is considered synonymous with eastern blue stem or streak of the black raspberry, as described originally by Wilcox.

Mild and severe streak are compared as to symptoms, seasons of symptom expression, and host ranges. Several of these symptoms indicate a close

relationship existing between the two diseases; others serve as a diagnostic means of distinguishing between them.

Notes on the known distribution of mild streak and varietal susceptibility to the disease are given.

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NOTABLE OUTBREAKS OF CRANBERRY FRUIT ROTS IN MASSACHUSETTS

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(Accepted for publication March 14, 1932)

THE MASSACHUSETTS CRANBERRY CROP OF 1931

Judged by the high standards of excellence prevailing among those who grow and handle Massachusetts cranberries, the crop of 1931 was of unusually poor keeping quality. In the opinion of some observers it was inferior even to the notorious crop of 1914. An idea of the relative amount of loss from decay during recent years may be gained from a comparison of the average amount of decay in storage lots from representative bogs in Plymouth County during the last 4 years (Table 1). The figures given are for the condition on November 15, when the fruit had been in storage approximately 2 months.

The Early Black is the most important variety in Massachusetts and makes up approximately half the total production. The Howes is second in importance and is the standard late variety. Eight to 10 lots were stored for each variety.

TABLE 1.—*Average condition of test storage lots of cranberries expressed as percentage of rotten berries, November 15*

Variety	Percentage of rotten berries, November 15			
	1928	1929	1930	1931
Early Black	7.4	11.0	13.1	31.0
Howes	6.5	9.0	6.7	14.1
Odd varieties	12.2	18.0	15.6	30.5

OTHER RECENT CRANBERRY CROPS WITH UNUSUAL LOSSES FROM FRUIT ROTS

Fruit rots of cranberries are, as is well known, caused by various fungi. Since, in general, the fungi concerned are indistinguishable in their effect on the fruits and are controlled by the same methods, they must for practical purposes be studied as a group.

In the case of the cranberry crop of eastern Massachusetts, there exists an unusually complete record of the relative amount of fruit rots for crops from 1912 to date. These are preserved in the reports of a cooperative sales company that handles a large portion of the crop of the state.

The existence of these records, together with weather observations taken in the immediate vicinity and in close proximity to a cranberry bog, made

possible the initiation of a study of the relation between the keeping quality of cranberries and prevailing weather conditions. This work, begun in 1922, resulted in an attempt to predict the keeping quality of individual crops. These predictions have been made for 9 years with such success that they have become a part of the regular commercial practice of the region and may be said to have passed the experimental stage.

As this work has been briefly discussed elsewhere[1] and a detailed account is to be published later, no reference is here necessary further than to say that the keeping quality of the crop seems to be correlated with the temperature during May and June and the frequency of rainfall during July and August. In general, it appears that unusually high temperatures during May and June, the period before the blossoms open, tend to be unfavorable to good keeping quality in the crop. Also, especially in the late varieties, unusually frequent rainfalls during July and August, the season during which the fruit is growing, apparently tend to favor an increase in decay. A combination of warm weather in May and June with frequent rainfall during July and August is apparently the most unfavorable condition of all.

The chart (Fig. 1) is designed to show the combined influence of temperature during May and June and frequency of rainfall during July and August. The abscissas represent temperature in May and June, here computed as a summation of day degrees above 50, and the ordinates the number of days with 0.01 in. or more rain during July and August. The heavy lines indicate approximately the normals. The upper right-hand portion thus includes those years having cool springs followed by summers with less than the average number of days with 0.01 in. or more rain and the lower left-hand portion those years having unusually warm springs followed by summers with more than the average number of days with 0.01 in. or more precipitation.

The abundance of fruit rots or, in other words, the keeping quality of the Howes variety each year, is indicated by symbols. Black dots indicate the crops having the largest losses from fruit rots. Circles with cross hatching indicate crops with keeping quality below average. Circles with F indicate fair quality, and unshaded circles, crops with slight losses from fruit rots. A similar chart based on the keeping quality of the Early Black variety shows a somewhat larger number of years above average quality but no other difference.

It will be noted that the 3 very bad years of record, 1914, 1922, and 1931, all fall in the group having warm May and June and more than ordinary number of days rain in July and August, and that the 3 other years

[1] Stevens, N. E. Cranberries used in trial forecasts as to keeping quality. U. S. Dept. Agr. Yearbook **1927**: 238–240, 1928 (and earlier papers on the same subject).

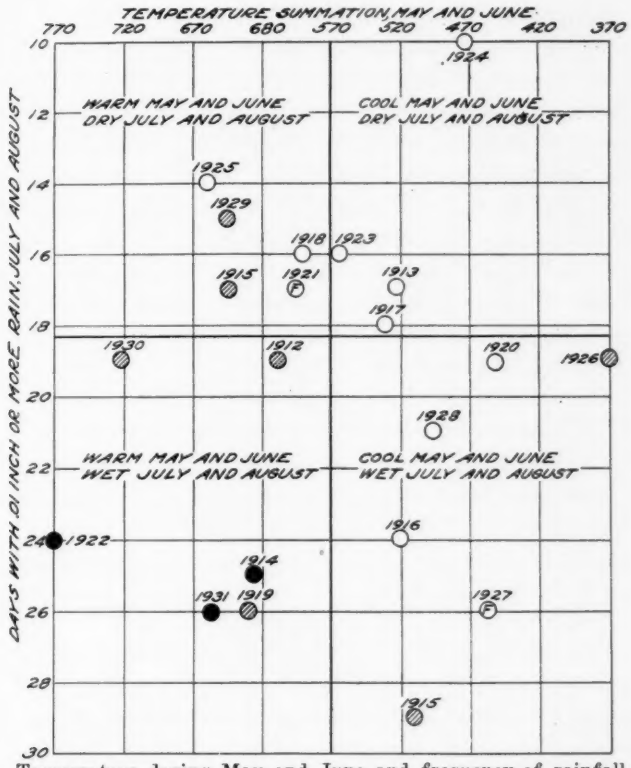

FIG. 1. Temperature during May and June and frequency of rainfall during July and August at East Wareham, Mass., 1912 to 1931, and the relative abundance of fruit rots in cranberries of the Howes variety in the Massachusetts crops of those years. Black dots, exceptionally large losses from fruit rots; circles with cross-hatching, losses from fruit rots larger than normal; circles with F, fair keeping quality; unshaded circles, slight losses from fruit rots.

in this group all had poor keeping quality. On the other hand, in the group having warm May and June but relatively dry July and August, there are 2 poor, 1 fair and 2 good crops, and in the group having a cool spring but frequent rain during July and August, 2 poor, 1 fair, and 3 good years. The 4th group, that having a cool spring and relatively dry July and August, on the other hand, contains thus far only years having crops of very high keeping quality.

EARLY RECORDS OF UNUSUAL LOSS FROM CRANBERRY FRUIT ROTS IN MASSACHUSETTS

Although the systematic study of plant diseases began in this country about 50 years ago and publications in this branch of botany have appeared in constantly increasing volume since 1886 or 1887, there is a surprising

dearth of information regarding the extent and severity of outbreaks of plant diseases, particularly diseases of minor crops. In attempting to trace the history of diseases of many crops, the student is too often driven to searching horticultural and agricultural magazines for chance references. Such records of diseases as exist are frequently in unpublished notes. So rare, indeed, are such records in available literature that almost any authentic record of a disease outbreak prior to 1900 deserves publication for its possible value to pathologists of to-day. The Plant Disease Survey project of the Bureau of Plant Industry was organized partly to provide a centralized unit where such records of diseased outbreaks could be recorded and made permanently available. Its records are, however, still fragmentary, and, of course, largely recent.

The interest aroused by the unusual amount of decay in the cranberry crop of 1931 led, first, to a search for records of previous crops of equally poor keeping quality and, second, to the study of weather records to determine, if possible, the frequency of the occurrence of weather that might have been expected to produce poor keeping quality. For obvious reasons unusual abundance of diseases is more likely to be made a matter of record than unusual freedom from disease. That is, a crop of poor quality is more likely to be recorded than one of high quality.

Such records of unusual losses from rot of cranberries prior to 1912 as could be found are in an early paper by Halsted and in the Proceedings of the American Cranberry Growers' Association, which, although its headquarters and most of its officers were in New Jersey, at that time numbered among its members growers from other States and maintained a lively interest in the crops of those states. From the records of this Association, it is evident that the crop of 1889 was of exceptionally poor keeping quality, both in New Jersey and Massachusetts, much like the more recent years of 1914 and 1931. 1889 was the year in which Byron Halsted was appointed botanist and horticulturist at the New Jersey Agricultural Experiment Station and took up a study of cranberry "scald." Early in his work he circularized a number of cranberry growers in Massachusetts and New Jersey and from his published record[2] it appears that the cranberry crop was considered of poor keeping quality in Massachusetts and Connecticut in 1883, 1887, and 1888, though apparently not so bad as in 1889.

The chart (Fig. 2) shows the distribution of the various years as to the weather conditions already discussed; that is, the temperature in May and June, here expressed as the sum of the monthly mean temperatures, and the number of days with 0.01 in. or more rain in July and August during the

[2] Halsted, B. D. Some fungus diseases of the cranberry. Part II. The cranberry scald. New Jersey Agr. Exp. Sta. Bul. 64: 16–40. 1889.

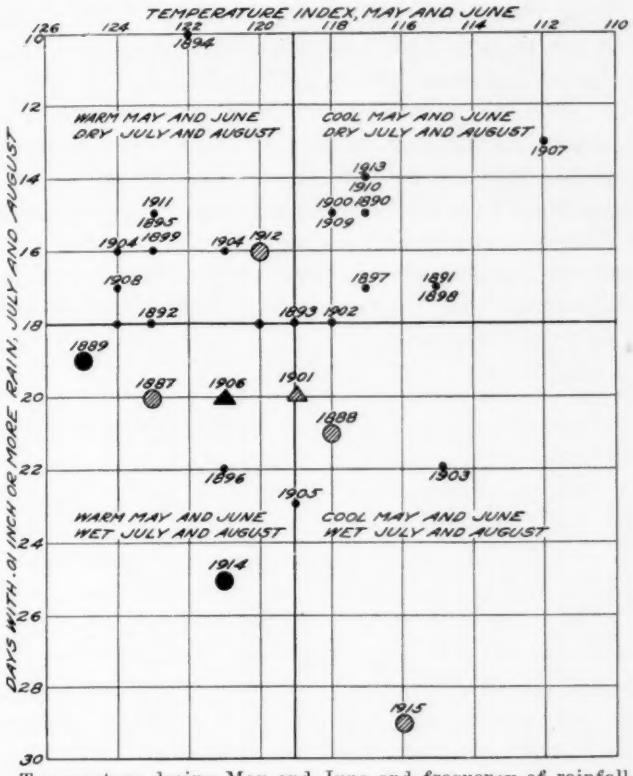

FIG. 2. Temperature during May and June and frequency of rainfall during July and August at Middleboro, Mass., 1887 to 1915, and relatively abundance of fruit rots in cranberries so far as records are available. Circular symbols same as in fig. 1; black triangle, very poor in New Jersey and no record for Massachusetts; triangle with cross-hatching, poor in New Jersey and no record for Massachusetts; small black dots, no record of unusual bad keeping qualities in either New Jersey or Massachusetts.

period 1889 to 1915, the period during which regular weather observations were made at Middleboro, Massachusetts. This weather observation station, while not located near a cranberry bog, as is that at East Wareham, was, during the period indicated, the nearest to the main cranberry-producing area. During this period 5 years, 1887, 1889, 1896, 1906, and 1914, fell within the group having weather believed to favor the increase of rot-producing fungi. Of these 5 years, 2, 1889 and 1914, are known to have had crops of very poor keeping quality, and 1, 1887, a crop of poor quality. The other years known to have produced crops of poor quality, 1888, 1912, and 1915, fell in the group having 1 unfavorable factor.

It would be of great interest if records could be obtained for the years 1896 and 1906, as well as for the 2 falling close to this group, namely, 1901

and 1905. Such records are not directly available, but what is known of the crops of those years in New Jersey furnishes indirect evidence that the losses from rot for the years 1901 and 1906 at least may have been larger than normal. While the cranberry crops of Massachusetts and New Jersey vary, as regards their keeping quality, to some extent independently of each other, it has been observed during the last 20 years that, when the crops of either State have been conspicuously poor in keeping quality, the crop of the other State has been below normal in this respect. It may then be worth while to note that the cranberry crop of New Jersey is known to have been of unusually poor keeping quality in 1901 and 1906.

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EFFECTS OF ANAEROBIC CONDITIONS ON THE GROWTH OF THE COTTON-ROOT-ROT FUNGUS, PHYMATOTRICHUM OMNIVORUM

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(Accepted for publication March 21, 1932)

INTRODUCTION

The cotton root rot caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar has been studied extensively since 1888 and is of recognized importance in the agriculture of the Southwest. Although much study, by various workers, has been given this disease, there still are numerous questions relating particularly to the physiology of the fungus that have not been investigated. Among these is a more thorough knowledge of the factors necessary for optimum growth of the fungus in the soil. It has been claimed by some (8) that the heavy black-waxy soils of Texas, being poorly aerated, furnish favorable conditions for the growth of the fungus, and others have reported that deep tillage (1, 2, 3), clean fallows (5), or fallow treatments combined with subsoiling (4), in some cases, have greatly reduced the infestation in cotton fields. Deep-seated infestations of the fungus in tree roots buried below the plow sole also have been reported (6, 7).

Although it is generally recognized that most organisms, excepting certain anaerobic bacteria and fungi, can live for only a short time unless supplied with free oxygen, yet little is known regarding the behavior of normally aerobic fungi under anaerobic conditions.

In order to gain further information regarding these points, the writers have made comparisons between the growth of the root-rot fungus in normal atmosphere, in an atmosphere of nitrogen, and in the presence of varying amounts of carbon dioxide.

MATERIALS AND METHODS

An isolation of *Phymatotrichum omnivorum* obtained from San Antonio, Texas, was used in these experiments. After uniform growth was established on neutral carrot agar, transfers of equal sizes were made to 5 Petri dishes and placed in a desiccator in which normal atmosphere was replaced with nitrogen by the pyrogallic acid method; the desiccator, with the enclosed cultures of *P. omnivorum,* being sealed by means of petrolatum and incubated at 29° C. for 8 days, together with 5 checks.

Further studies were conducted by growing the fungus in 25, 50, 75, and 100 per cent carbon dioxide in an anaerobic culture jar, with 50 cc.

Erlenmeyer flasks instead of Petri dishes as the containers for the organism. This change was necessary in order to reduce the possibilities of contaminating the cultures. The carbon dioxide was generated by adding hydrochloric acid to marble. Then the gas was bubbled through water and caught in a graduated container. A layer of lubricating oil, $\frac{1}{2}$-in. thick, was used in separating the water from the carbon dioxide in this container. After generating a container full of the gas it was forced into the anaerobic jar through rubber tubing by admitting water to the carbon dioxide container below the oil layer. As water was added, the oil layer would rise and force the carbon dioxide into the anerobic jar. This method permitted the measurement of the volume of gas used and the calculation of the approximate concentrations employed.

RESULTS

The results obtained in growing the fungus in normal atmosphere, in an atmosphere of nitrogen, and in varying concentrations of carbon dioxide are given in tables 1 and 2; and the effect of aerobic and anaerobic conditions upon growth after a period of 8 days is illustrated in figure 1.

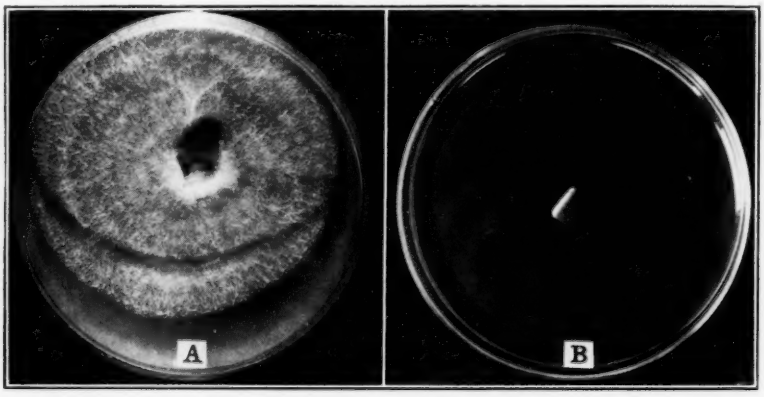

FIG. 1. Growth of *Phymatotrichum omnivorum* after 8 days on neutral carrot agar. A. In normal atmosphere. B. In an atmosphere of nitrogen.

TABLE 1.—*Growth of Phymatotrichum omnivorum under aerobic and anaerobic conditions*

Atmosphere	Diameter of colonies in millimeters[a]					
	Petri dish number					
	1	2	3	4	5	Ave.
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
Nitrogen	0.0	0.0	0.0	0.0	0.0	0.0
Check	80.0	81.0	80.0	83.0	82.0	81.1

[a] Measurement recorded at the end of 8 days.

TABLE 2.—*Effect of carbon dioxide on the growth of Phymatotrichum omnivorum*

Atmosphere	Amount of CO_2	Diameter of colonies in millimeters[a]					
		Flask numbers					
		1	2	3	4	5	Ave.
	%	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
Carbon dioxide	25.0	45.0	42.0	43.0	44.0	42.0	43.2
Check		45.0	45.0	44.0	45.0	44.0	44.6
Carbon dioxide	50.0	22.0	28.0	25.0	22.0	29.0	25.2
Check		45.0	44.0	45.0	40.0	43.0	43.4
Carbon dioxide	75.0	10.0	11.1	11.1	11.1	10.0	10.7
Check		45.0	46.0	45.0	46.0	47.0	45.8
Carbon dioxide	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Check		45.0	44.0	46.0	45.0	45.0	45.0

[a] Measurements recorded at the end of 5 days.

DISCUSSION AND SUMMARY

The data reported show that growth of *Phymatotrichum omnivorum* is inhibited by anaerobic conditions and is notably restricted by concentrations of carbon dioxide greater than 25 per cent Although the fungus does not grow in an atmosphere of 100 per cent nitrogen or carbon dioxide, it is not killed and, soon after being returned to aerobic conditions, growth occurs.

How long this organism is able to survive under anaerobic conditions is not known, but further experiments are being made.

The aerobic requirements for growth of the fungus, as indicated by these experiments, may explain the differences in root-rot infection previously referred to in this paper, which have been observed in cotton fields following such treatments as subsoiling, or fallowing, or fallow combined with deep tillage.

Subsoiling infested fields during the late summer or early fall may provide the necessary aeration for the germination of the sclerotia, thereby lessening the chances of winter survival of the fungus.

The recurrence of the disease in areas that have been kept in clean fallow for 3 and 4 years may be due to anaerobic conditions of the subsoil, especially in the highly calcareous, black-waxy lands of Texas, as well as to deep-seated infections of the fungus in tree roots below the plow sole. The sclerotia of the fungus, found frequently below the ordinary depth of cultivation in these soils, may remain dormant for long periods until sufficient

oxygen to stimulate their germination is provided either by the growth of plant roots, by subsoiling, or by deep natural cracking of the soil as occurs in dry seasons.

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PHYTOPATHOLOGICAL NOTES

The Rôle of Diseases in Plant Quarantines.—A circular, issued periodically by the Bureau of Plant Quarantine of the United States Department of Agriculture, lists 42 Federal plant quarantines and orders, of which 22 are foreign and 20 domestic. Of the 22 foreign quarantines, which restrict or prohibit the importation of plants and plant products from foreign countries, 7 are maintained because of diseases alone, 9 because of insects alone, and 6 because of both insects and diseases.

Of the 20 domestic quarantines 4 are imposed because of diseases alone: Black Stem Rust (Quarantine No. 38); White Pine Blister Rust (No. 63); Woodgate Rust (No. 65); and Phony Peach (No. 67). Two domestic measures, the quarantine on Sugarcane (No. 16) and that on Narcissus Bulbs (No. 62) is based on both insects and diseases.

Plant diseases thus constitute an essential feature in 6 of the 20 domestic quarantines and in 13 of the 22 foreign quarantines; altogether, plant diseases are a fundamental part of 19 of the 42 Federal quarantines.

The 7 foreign plant quarantines, based on diseases alone, may be summarized as follows:

Quarantine No. 3, the Potato Wart Quarantine, forbids the importation of potatoes from specified countries and localities known to have potato wart.

No. 7, the White Pine Blister Rust Quarantine, established to prevent the entrance of this disease, forbids the importation of 5-leaf pines and *Ribes* and *Grossularia* spp. from parts of the world where the disease is known to occur.

No. 19 prohibits the entrance of nursery stock, buds, and scions of citrus from the world at large because of citrus canker and other diseases.

No. 24. By this quarantine the importation of corn and related plants in any stage (except corn as seed) from certain Asiatic areas, the Pacific islands, and Australasia is prohibited because of the Physoderma diseases and downy mildews (*Sclerospora* spp.). Shelled corn may enter from these countries under strict regulation.

No. 28 aims to prevent the further entrance of citrus canker by excluding all citrus fruits except oranges of the mandarin class from the general Asiatic and Pacific island regions, and South Africa, excepting Australia, Tasmania, and New Zealand.

No. 34. Seeds, plants, and cuttings of bamboo are prohibited entry from all parts of the world in order to keep out numerous diseases affecting bamboos, especially the bamboo smut, *Ustilago shiraiana.*

No. 59, Flag Smut. All species and varieties of wheat, unless milled or processed, are forbidden entry from countries where flag smut is known to exist.

Foreign quarantines in which injurious diseases are coupled with insects as a basis for the measures taken are as follows:

No. 15 aims to protect sugar-cane culture in the United States by excluding all living canes or living cuttings or parts of sugar-cane.

No. 37 covers nursery stock, plants, and seeds from all countries and provides for the regulated entry of such of these as may be imported.

No. 41. Entry of Indian corn and related plants from all foreign countries is made subject to regulations on account of the European corn borer and other dangerous insects and plant diseases.

No. 55 is concerned with seed or paddy rice, the unhulled and unpolished grain used for seed. Such seed rice is not permitted entry, except under regulation from Mexico.

Potato Regulations. A special set of regulations has been issued to govern the importation of potatoes from all countries from which they are not excluded by Quarantine No. 3.

Plant Safeguard Regulations. These regulations are intended to provide means for the safe handling of restricted or prohibited plants and plant products brought into a port of entry for reexport to some other country or as ships' stores or other case of temporary stay. Such materials may be safeguarded against the escape of pests under these regulations while they remain in United States territory.

It is noted that in connection with all quarantines of a prohibitory nature special provision is made in the Plant Quarantine Act for importations by the Department of Agriculture, a feature that enables the Department to introduce under safe conditions desirable new varieties, rare plants, useful crop plants, or experimental materials.—W. A. MCCUBBIN, Bureau of Plant Quarantine, Washington, D. C.

The Occurrence of the Perfect Stage of Phomopsis mali in the United States.—The importance of apple-foliage diseases of the frog-eye type in the fruit-growing sections of northwest Arkansas was stressed by Scott and Rorer[1] in 1907. These investigators found that the leaf-spot diseases were responsible for premature defoliation, small fruit of poor quality, weakened trees, immature fruit buds, and the reduction of the crop in succeeding years. While the general recognition by the orchardists that a definite spray program must be followed every year for the control of apple diseases has doubtless reduced the importance of apple-foliage diseases in recent years, the fact remains that in many orchards apple-leaf spots still are present every year. In some years they become so numerous that they produce injuries almost as severe as those observed by Scott and Rorer.

During the growing season of 1931 the writer repeatedly secured cultures of a species of Phomopsis from apple-leaf spots from orchards in the

[1] Scott, W. M., and J. B. Rorer. Apple leaf spot caused by Sphaeropsis malorum. U. S. Dept. Agr., Bur. Plant Indus. Bul. 121: 47–54. 1907.

vicinity of Fayetteville, Arkansas. A comparison of these cultures with type material has demonstrated that they are identical with *Phomopsis mali* Roberts.

On November 6, 1931, one of these cultures, which had been transferred to sterile apple twigs in test-tubes on August 8, was found to have several long beaks or neck-like structures projecting above the surface of a twig. These structures were found to be the beaks of perithecia that had formed beneath groups of pycnidia. Following the discovery of these perithecia other cultures of *Phomopsis mali* were transferred to sterile apple twigs and perithecia eventually formed in 10 different isolations out of a total of 34 tested.

A study of the morphology of the perithecia, asci, and spores indicated that the fungus was a member of the genus Diaporthe.

Single ascospore isolations were made from various perithecia and transferred to potato-dextrose agar. Pycnidia formed on the agar and conidia began to ooze from them in 28 days. An examination of the pycnidia and the spores showed them to be identical with those of *Phomopsis mali*. After 90 days a new generation of perithecia, with typical Diaporthe characters, appeared in these cultures. Similar results were secured when ascospores were transferred to sterile apple twigs in test-tubes, but the rate of development was accelerated, conidia appearing in 19 days and perithecia in 54 days. The cultures from ascospores gave rise to *P. mali* conidia that, when transferred to sterile apple twigs, produced cultures in which perithecia appeared after 76 days.

It is evident, as the result of these experiments, that a genetic connection exists between *Phomopsis mali* and the Diaporthe and, conversely, that the Diaporthe is the perfect stage of *P. mali*.

In the apple-twig cultures the globose to globose-flattened perithecia are embedded in a matrix of fungus hyphae, pycnidia, and twig tissues. The perithecia occur singly or in groups but are always separately erumpent by curved, twisted, hairy, black (except at the extreme tip) beaks 1 to 4 mm. long.

The outer wall of the perithecium is black and membranous, enclosing an inner layer of brown tissue that surrounds the asci. The asci are approximately 40 to 60 by 5 to 7 μ, containing eight 2-cell, hyaline spores measuring 9.5 to 13.5 by 2.7 to 3.8 μ. The ascospores are obtuse at both ends, slightly constricted at the septum, and each cell contains 2 oil drops. No paraphyses were observed.

Kidd and Beaumont,[2] after a comparison of American and European cultures of Phomopsis, decided that *Phomopsis mali* is probably the imper-

[2] Kidd, M. N., and A. Beaumont. Apple rot fungi in storage. Trans. Brit. Mycol. Soc. 10: 98–118. 1924.

fect stage of *Diaporthe perniciosa* Marchal. The morphological characters of the ascomycete that developed in the writer's cultures of *P. mali* agree very well with the published descriptions of *D. perniciosa,* and the fungus may be referred to this species pending a critical study of European cultures and *exsiccati.*—JOHN C. DUNEGAN, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the Department of Plant Pathology, University of Arkansas.

Another Gall-Forming Bacterium.—In the spring and summer of 1932, a soft nodular-type gall was found destroying grafted plants of *Gypsophila paniculata* Linn. (baby's breath) in a New Jersey nursery. The galls occurred at the crown and root in the region of the graft; none was found on the aerial stems. In general appearance most of the galls were not so globular as those of the typical crown ball produced by *Bacterium tumefaciens* Sm. and Town. but usually produced a flat nodular growth similar to the outgrowths of the pocket disease of sugar beets produced by *Bact. beticola* (Sm., Br., Town.) Poteb.

The Gypsophila galls ranged from ½ cm. to 3 cm. in diameter (Fig. 1), but there was evidence of gall decay in some plants, which indicated the

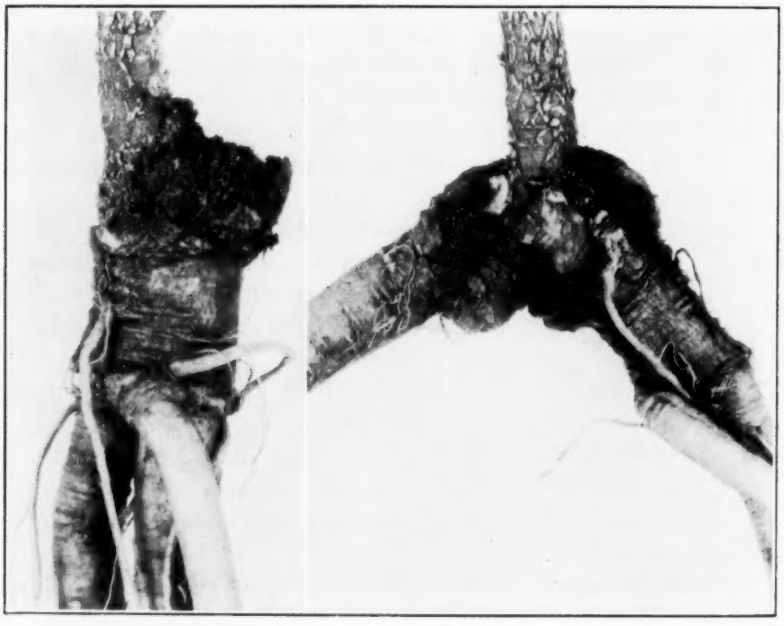

FIG. 1. Galls on Gypsophila (baby's breath) from a nursery in New Jersey. ×1. Photographed on May 25, 1932.

existence of still larger galls. When cut across, the tumors were sound, creamy white, with water-soaked areas, a condition unlike crown gall, olive gall, or pocket disease of sugar beets. The two latter types have pocket-like areas in mature tumors filled with a gummy substance or from which a slimy ooze exudes, depending on the age and condition of the gall. Crown-gall tissue on most hosts, when sound, is white and without water-soaked areas.

The organism isolated from the Gypsophila gall is a yellow one and its infectious nature was proved by inoculations into the crown and root of Gypsophila plants. In less than 2 weeks after inoculation definite outgrowths developed at the crown and in 2 months the tumors were 2½ cm. across. Inoculations made into the aerial stems of Gypsophila plants produced cankers instead of galls. Three different sets of inoculations made into sugar beets of different ages failed to produce infection, as did inoculations into several other plants, such as Ricinus, Bryophyllum, tomato, and garden balsam.

The organism can grow at very low and high temperatures for a plant pathogen; it is a motile rod whose flagella have been stained and found to be bipolar, and it has been recovered from the galls produced by inoculations.

So far as the study has progressed, the organism appears to be another gall-forming bacterium, but considerable comparative cultural and morphological work needs to be done to be sure it is not related to the yellow, tumor-forming sugar-beet organism, *Bacterium beticola*.—NELLIE A. BROWN, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

A Convenient Method of Bleaching and Clearing Leaves.—In the course of a program of histological studies of the genus Ribes L., it was found necessary to bleach and clear a large number of leaves preparatory to determining the character, frequency, and distribution of stomata, trichomes, hydathodes, and crystal druses. A commercial grade of sodium hypochlorite, with or without the addition of sodium hydroxide (flaked lye), has proved more convenient and satisfactory than any of the reagents commonly used for this purpose.

In brief, the procedure followed consisted in processing leaf tissues in air-tight containers, such as corked shell vials or small screw-top glass jars, containing an aqueous solution of commercial sodium hypochlorite and lye, until sufficiently bleached and cleared for microscopic examination.

The most satisfactory concentrations of the two substances in the solution range from 5 to 20 per cent by volume of sodium hypochlorite and from 1 to 5 per cent by weight of sodium hydroxide, depending upon the

type of material under investigation. A solution of 10 per cent commercial sodium hypochlorite and 2½ per cent sodium hydroxide gave satisfactory results in many cases, but a solution containing 20 per cent of the hypochlorite and 5 per cent sodium hydroxide proved more efficient. The latter solution was the one most frequently used and was sufficiently strong to bleach and clear all leaves that were worked upon. A solution of 10 per cent hypochlorite and 5 per cent sodium hydroxide, however, is recommended for preliminary work with unfamiliar material. The presence of an appreciable quantity of sodium hydroxide hastens the process, results in clearer materials and increases the ease with which the leaves can be "wetted" in the solution.

The time required for clearing the leaves varies from 12 to 96 hours, depending upon the type, size, and condition of the leaves and the strength of the solutions used. The solution seems to penetrate the leaf through cut edges, or other breaks in the epidermal layers, and not directly through uninjured tissues. Consequently, small pieces of leaf tissue require less time to be processed than whole leaves. In some cases, one or more changes of the processing solution may be necessary.

While the leaves are in the clearing and bleaching solution, some maceration may be expected. It may therefore be necessary to pour them into a large pan of water and to straighten them out upon a submerged piece of glass before undertaking microscopic examination. Whole leaves or large pieces of leaf tissues are conveniently manipulated for observation on the cover glasses of lantern slides.

Commercial sodium hypochlorite appears to keep indefinitely in the bottle in which it is sold as long as the bottle remains tightly stoppered. Diluted solutions do not seem to keep particularly well, but, when made, should be stored in dark, tightly-stoppered bottles in a cool place.

The process is equally satisfactory for fresh material, dried (herbarium) material and for material preserved in formalin-acetic alcohol, but cannot be used for material to be imbedded in paraffin, because the cell contents are dissolved by the solution and all details are lost. This process has been used on the leaves of a variety of California plants including several Ribes species, which, as a rule, are quite tanniniferous; and upon *Arctostaphylos patula* Greene, green manzanita; *Myrica californica* Cham., wax myrtle; and *Castanopsis sempervirens* Dudley, bush chinquapin, all of which bear what might be termed durable leaves.

The method given herein is, of course, intended for temporary mounts. In all microscopic work on the leaves bleached and cleared in the manner described, weak illumination has been found to be more satisfactory than strong illumination.—CLARENCE R. QUICK and FRANK A. PATTY, *Division of Blister Rust Control, Bureau of Plant Industry, United States Department of Agriculture.*

The Nonspecificity of the Brown-Ring Symptoms in Narcissus Attacked by Nematodes.—The purpose of this note is to call attention to the fact that the brown-ring symptoms of narcissus bulbs, if of true or apparent nemic origin, are not specific for *Tylenchus dipsaci* Kühn. According to our observations, various other species, such as *Aphelenchoides fragariae* Ritzema Bos,[1] *A. parietinus* (Bastian), *Aphelenchus avenae* Bastian, and even *Cephalobus striatus* deMan, may produce or be connected with brown rings. Development and character of the symptoms are in all instances very similar (Fig. 1, A–D and legend), although none of the forms mentioned

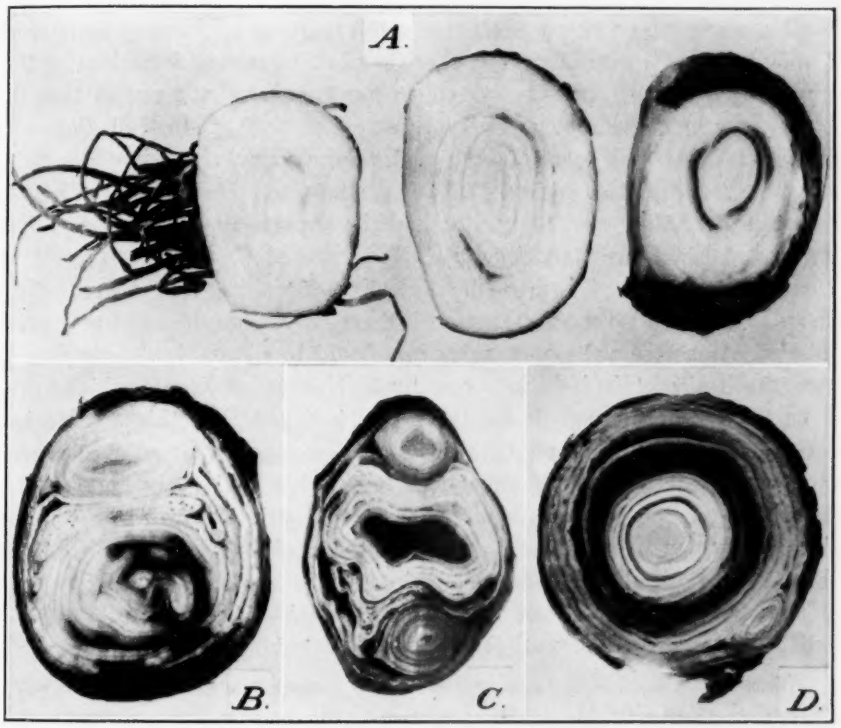

FIG. 1. A. Rather early stage of brown-ring symptoms produced by *Tylenchus dipsaci* in a narcissus bulb. The crosscuts from right to left show how the disease proceeds from the neck region of the bulb downwards. B. Brown rings produced by *Aphelenchoides parietinus*. *Aphelenchus avenae* produces very similar rings. C. Brown center in the neck region of a narcissus bulb containing large numbers of *Cephalobus striatus*. D. Brown rings produced by *Aphelenchoides fragariae*.

seems to be so virulent and of such high pathogenicity as *T. dipsaci*. In narcissus bulbs affected by any of these nemic forms the disease normally

[1] Synonyms of this form are: *Aphelenchoides subtenuis* (Cobb), *A. olesistus* Ritzema Bos, and *A. ritzema-bosi* Schwartz.

begins at the neck, the ring symptoms proceeding downwards. *Cephalobus striatus* was observed in two cases, in both of which only the center portion of the neck was affected, as shown in figure 1, C. It is possible that this species, which, according to our knowledge, acts more as a saprophyte, simply takes advantage of the weakened condition of the attacked region as sometimes produced by faulty hot-water treatment. With the exception of *T. dipsaci*, *Aphelenchoides fragariae* is most frequently found, of the forms mentioned.—G. STEINER and EDNA M. BUHRER, Division of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture.

Diaporthe citri (Faw.) Wolf, the Perfect Stage of Phomopsis citri and P. californica.—Study of perfect stages and of numerous isolations of these previously supposed different species[1,2] has convinced the writer that they should now be considered as one species. The perfect stage of *Phomopsis californica* Faw. has been studied[3] and no characteristics have been found that would distinguish it from the Diaporthe stage described by Wolf[4] for *P. citri* Faw. Moreover, the writer had the opportunity to examine a large number of isolations made by R. G. Tomkins, of Cambridge, from fruits from various parts of the world: Palestine, South Africa, Brazil, Spain, Florida, etc., and no characteristic differences that would seem to separate these isolations from those of California could be detected. The writer has also examined certain isolations sent by K. Nakata, of Japan, which appear to be the same fungus. It is, therefore, believed that this species is of world-wide distribution on Citrus. Melanose, the effect so prevalent in Florida and certain other countries, is believed to be largely dependent on climatic conditions, especially a proper combination of rainfall, temperature, and growth conditions. Although the fungus is present in climates like California and Palestine, producing stem-end rot, it is rarely evident as melanose. Melanose has been found occasionally in a mild form in California.—H. S. FAWCETT, Riverside, California.

[1] Fawcett, H. S. The cause of stem-end rot of citrus fruits (*Phomopsis citri* n. sp.). Phytopathology **2**: 109–113. 1912.

[2] Fawcett, H. S. A new Phomopsis of Citrus in California. Phytopathology **12**: 419–424. 1922.

[3] Fawcett, H. S., and C. L. Shear. Discovery of the perfect stage of a Phomopsis on lemon bark. (Abst.) Phytopathology **19**: 1138. 1929.

[4] Wolf, F. A. The perfect stage of the fungus which causes melanose of Citrus. Jour. Agr. Res. **33**: 621–625. 1926.

BOOK REVIEW

Barton-Wright, E. C. *Recent Advances in Botany.* 287 pp. 60 figs. P. Blakiston's Son & Co., Philadelphia. 1932. Price $4.00.

This volume, which aims to present only a certain few of the recent advances in botany, makes no reference to work in general cytology or genetics; the 9 chapters concern general morphology (1), paleobotany (1), taxonomy (1), mycology (3), algae (2), and virous diseases of plants (1).

In the chapter on general morphology special attention is given to the relation between size and form, the phyllode theory of the monocot leaf, and carpel polymorphism. The chapter on paleobotany deals especially with the carboniferous bryophytes of the genus Hepaticites Walton and the Caytoniales of the Mesozoic; this latter group opens up an entire new chapter in the history of the Angiosperms. An excellent thread to guide the reader through the maze of modern taxonomic literature is given in the chapter on the species problem, with emphasis on the conflicting claims of the habitat (ecospecies) and of the genetical composition (genospecies) in determining "species," with especial stress on the work of Turesson.

Chapter 4 reviews papers on the life histories of the various groups of fungi, with emphasis on the work of Cook and Schwarz on Plasmodiophora and of Wilson on *Reticularia lycoperdon.* Attention is also given the life histories of the Chytridiales, Kanouse's work on the classification of the Oomycetes, and some of the papers on the double nuclear fusion in the Ascomycetes. In regard to this last problem, the recent work seems to support the view that there are two nuclear fusions in the life cycle and that reduction is accomplished in two stages, contrary to the opinion of Claussen.

The remaining two chapters on the fungi are concerned with the problems of heterothallism and mycorrhiza. In addition to the original work of Blakeslee on the Phycomycetes, similar conditions have been reported by many workers in the Ascomycetes and Basidiomycetes. In this last group, the problem becomes especially complex because of the polyspory present in many forms. Most of the work has dealt with facts rather than with explanations, although Gwynne-Vaughan has attempted to explain heterothallism in the Ascomycetes and Hymenomycetes on the basis of nutrition. Assuming that certain strains (−) can extract certain food substances essential for fruiting body formation from the substrate, while other strains (+) can extract others equally essential, then the fruiting bodies would be formed only when the two strains met. If in "quadrisexual" forms, such as *Aleurodiscus polygonius* and *Coprinus lagopus,* the 4 characters are segregated in Mendelian fashion, the same explanation would hold.

The chapter on mycorrhiza is concerned chiefly with the work on the orchids, heaths, Bryophytes, and forest trees. The precise rôle of the endophytic fungi still remains to be cleared up, although the evidence points more and more in favor of the view that the fungi are of genuine benefit to the "host," either to hydrolyze carbohydrates (orchids), increase the N supply (heaths), or aid in getting mineral salts (trees). The double infection theory of Peyronel is discussed, according to which it is assumed that the infection is of a composite nature, an initial infection paving the way for a second (and different) form.

The two chapters on the algae are devoted to the Phaeophyceae and the Florideae, with emphasis upon their cytology and life histories in an attempt to straighten out the more or less confused taxonomic questions in these groups and to clarify the gametophyte-sporophyte relationships.

The last chapter (virous diseases) is doubtless of most interest to the readers of this journal and for this reason will be discussed in somewhat greater detail. Although these diseases have been known since 1892, only in the last decade has their study gained much headway. Since they are present in herbaceous more than in woody plants, the former group has received the most serious study (notably the Solanaceae). The disease is generally accompanied by chlorosis of the leaves and necrosis of the phloem. Although death seldom results, recovery occurs very rarely if ever. Storey has claimed the recovery of sugar-cane from the streak disease and Robbins of sugar beets from mosaic. In many of these cases, however, the symptoms have probably only been masked.

The viruses are, in general, very resistant to high temperatures and to most of the common antiseptics (acids, alkalis, alcohol, etc.). Formaldehyde was the most efficient in destroying the virus of tobacco mosaic, which withstands heating for 2 days at 80° C., simple storage for 5 years, and 90 per cent alcohol acting for 1 hr. Cucumber-mosaic virus is not so resistant; it loses its infecting power after heating up to 70°, after standing for 7 days, or when treated with 40 per cent alcohol. Other viruses range, for the most part, between these two.

No satisfactory system of classifying or naming these diseases has yet been developed. As many as 8 different viroses have been described for tobacco (Johnson) and 5 to 7 for the potato. Work is now being done on keys to distinguish the various kinds, which are thus far distinguished by different letters of the alphabet. Some workers have even anticipated the discovery of future similar virous diseases in specific hosts and have named a disease as "virus A" when there is as yet no "B." This the author considers to be the source of needless confusion.

The best work on the subject has been done on the question of transmission. Tobacco mosaic is spread very readily even by rubbing a diseased

plant with the fingers and then a healthy plant. Grafting invariably results in transmission, while transmission through the seed is comparatively rare (cucumber mosaic). In nature the chief agents are insects, for which the reader is referred to the summary by Kenneth Smith (Biol. Rev. 6). In potato leaf roll, aphids (*Myzus persicae*) are the chief carriers. In some cases one insect may transmit several diseases or one disease may be transmitted by several insects, while in other cases there is great specificity between disease and vector. In many cases, however, the transmission is not a simple mechanical transfer; a certain incubation time must elapse between feeding and transmission, which points to a biological rather than chemical nature of the disease.

Smith found in experimenting with tobacco and potato mosaic that there was a difference between the types of infection produced by needle inoculations and aphid transfers. In the latter case, there was little increase in virulence with successive inoculations such as occurred with the needle transfers. The aphid thus seems to fail to transmit some element of the virus that produces the more lethal and necrotic symptoms. The virus is thus divided into x and y elements. Work by Allard and others has shown that it is possible for a plant to be normal in appearance and still be a "carrier" of a virous disease.

The causative organism has been sought at various times in bacteria, protozoa, and mycetozoa, all of which were found in plants infected with virous diseases. Of late, attention has been centered on the X-bodies (Goldstein) associated with the protoplasm and generally near the nuclei in infected plants. These X-bodies are granular, deeply staining, vacuolate, and roughly spherical. Since they are more or less amoeboid, are proteinaceous, have been associated by some workers with mitochondria, and seem to increase and divide, they have been thought to be living entities, but this question remains to be settled. The work of Henderson Smith on the X-bodies in *Solanum nodiflorum* tends to show that, although of a protein nature, they are not organisms. At any rate, no plant virus has ever been made to multiply outside its host.

As to the physiology of the infected plants, comparatively little has been done. During the disease, carbohydrates accumulate in the leaf accompanied by the necrosis of the phloem. That the latter is not entirely the cause of the former, however, is shown by the fact that the accumulation of starch in leaves (potato roll) may occur *before* the necrosis of the phloem (Murphy). Translocation is the most difficult problem to explain in leaf-roll plants. Although no sucrose is found in the petioles, some sugars get down to the tubers, and it is concluded that there must be a slow leak of hexoses *via* the ground parenchyma of the petioles and stems. In the spike disease of

Santalum, the pH value is lowered in diseased leaves, which were also found (Iyengar) to be deficient in K and Ca. Diastase activity, on the other hand, is greater in diseased than in healthy leaves.

In most cases the virus seems to move in the plant in the phloem, as evidenced by ringing and similar experiments, but Caldwell claims that under exceptional conditions aucuba virus in tomatoes may be made to travel in the xylem but is incapable of leaving it to infect the living tissues. Only when the leaves were crushed and the virus could escape from the conducting vessels was infection possible. Rate of movement of "curly-top" virus in the sugar beet was found by Severin to be 10 cm. per hour, while Storey found the virus of corn mosaic to travel at 10 to 20 cm. per hour.

This little volume will be found an extremely valuable addition to the library of the nonspecialist who wishes to keep pace with the progress in related fields. In a work of this nature, the materials must be selected with great care and presented critically to be of the greatest value. In this regard the author has done a good piece of work. Furthermore, each chapter is prefaced with sufficient introductory, historical matter to make the following discussion intelligible. In spite of such minor slips as on page 273, where pH value and H-ion concentration are considered synonymous, the book is well written and quite up to the high standard of the "Recent Advances" series of which it is a part.—ORAN RABER, Biological Abstracts, Washington, D. C.

THE PRINCIPLES OF PLANT QUARANTINE

INTRODUCTION

For many years a definite need has existed for a simple but adequate statement setting forth the principles that ought to be followed in the establishment and enforcement of plant quarantines. If such a statement could be prepared so as to embody the best opinion available on the subject it would serve as a valuable guide in practice in a field that has developed ever-increasing complexity. In 1926 the National Plant Board undertook the preparation of such a statement. In succeeding years the original draft has been subjected to critical revision, both by the National Plant Board itself and by members of the 4 regional plant boards. From this process of revision the statement of quarantine principles may be said to have emerged with the approval of the quarantine and regulatory representatives of all 48 States. As thus finally approved it was issued July 25, 1931, by the National Plant Board, under the title, "Principles of Plant Quarantine." As a valuable contribution in the regulatory field it is reproduced for the information of plant pathologists, who logically must share with entomologists a national responsibility for the wise future development of plant quarantines.

PRINCIPLES

1. **Definition.** A quarantine is a restriction, imposed by duly constituted authorities, whereby the production, movement or existence of plants, plant products, animals, animal products, or any other article or material, or the normal activity of persons, is brought under regulation, in order that the introduction or spread of a pest may be prevented or limited, or in order that a pest already introduced may be controlled or eradicated, thereby reducing or avoiding losses that would otherwise occur through damage done by the pest or through a continuing cost of control measures.

2. **Basis in Logic.** Since the ends to be attained by a quarantine and the measures required by it could not be undertaken by private individuals or groups, involving as they do restrictions on areas, persons, or activities for the benefit of wider interests or the public at large, resort to regulation imposed by public authority is logical.

3. **Necessity.** Establishment of a quarantine should rest on fundamental prerequisites, as follows: (1) the pest concerned must be of such nature as to offer actual or expected threat to substantial interests; (2) the proposed quarantine must represent a necessary or desirable measure for which no other substitute, involving less interference with normal activities, is available; (3) the objective of the quarantine, either for preventing introduction or for limiting spread, must be reasonable of expectation; (4) the economic gains expected must outweigh the cost of administration and the interference with normal activities.

4. Legal Sanction. A quarantine must derive from adequate law and authority and must operate within the provisions of such law.

5. Validity. A quarantine established for the purpose of attaining an objective other than that which it indicates or defines is open to serious criticism, even though the actual objective is itself desirable.

6. Public Notice. If the circumstances will permit, public notice of a proposed quarantine should be given and those interested should be invited to contribute facts in their possession. But if the objective would be defeated by the delay required for such notice and discussion, duly-constituted authorities should assume responsibility for the decision to impose or withhold quarantine action.

7. Scope. The extent of restrictions imposed by a quarantine should be only such as are believed necessary to accomplish the desired end, but on the other hand the objective of a quarantine should not be jeopardized by omission of any necessary restriction.

8. Relation to Eradication. If a quarantine is imposed in order that eradication of a pest from a given area may be undertaken, the restrictions involved may properly be relatively extensive, because of the importance of the objective sought, and because the time through which the quarantine will operate may be expected to be relatively limited.

9. Relation to Retarding Spread. If a quarantine is imposed for the purpose of limiting or retarding spread of a pest, but without expectation of eradication, the restrictions imposed should be such as are in line with the objective of the quarantine and should recognize the fact that continuance of the pest in the area where it is established, or possibly its spread in time to new areas, is accepted.

10. Cooperating Authorities. Since quarantines usually involve relations between public authorities, such as those of the government of one country with that of another, or of Federal and state governments, or of state government and local authorities, the cooperative relationship that is necessary to adequate enforcement should be clearly recognized and duly provided for.

11. Cooperation of the Public. Because of the fact that the success of a quarantine requires that its restrictions be fully maintained, it is essential that all persons who are affected by it adhere to its requirements. In order that this end may be attained the administration of a quarantine should seek the intelligent cooperation of the public affected, rather than depend exclusively on police powers, the imposition of penalties, or resort to court action.

12. Clarity. In order that a quarantine may be administered readily and consistently, it should be designed with care, should be phrased clearly, and should be made as simple as is consistent with legal requirements and the objective to be attained.

13. Information Service. Since the persons affected by a quarantine may not reasonably be expected to possess full or accurate knowledge of the circumstances that make it necessary, or the nature and importance of the

aim sought, and since compliance with quarantine restrictions will be more complete if the objective and plans are understood, measures should be taken to set forth the conditions existing, the means to be employed, and the end to be attained, and these measures should be continued from time to time as the undertaking proceeds toward accomplishment.

14. Research. If an emergency requires the establishment of a quarantine before satisfactory biological data are available, provision should be made as soon as possible for extending the fund of biological knowledge. The authority that exercises the right to establish a quarantine should command or secure the means for biological research, both in order that the quarantine may be made more efficient, and in order that the restrictions may be lessened where possible. The need for resarch, however, should not be permitted to delay the establishment of a quarantine believed by authorities to be desirable, thereby jeopardizing the objective that might otherwise have been attained.

15. Modifications. As conditions change, or as further facts become available, a quarantine should promptly be modified, either by inclusion of restrictions necessary to its success or by removal of requirements found not to be necessary. The obligation to modify a quarantine as conditions develop is a continuing obligation and should have continuing attention.

16. Repeal. If a quarantine has attained its objective, or if the progress of events has clearly proved that the desired end is not possible of attainment by the restrictions adopted, the measure should be promptly reconsidered, either with a view to repeal or with intent of substituting other measures.

17. Notices to Parties at Interest. Upon establishment of a quarantine, and upon institution of modifications or repeal, notices should be sent to the principal parties at interest, especially to Federal and state authorities and to organizations representing the public involved in the restrictive measures.

NATIONAL PLANT BOARD,
OFFICE OF THE CHAIRMAN, W. C. O'KANE,
DURHAM, NEW HAMPSHIRE, JULY 25, 1931.

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